Novel Detection and Removal of Hazardous Biocide Residues Historically Applied to Herbaria

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ABSTRACT

This research is concerned with the detection and removal of hazardous biocide residues from historic applications to herbarium collections. There are two main aims:

- To develop a rapid, cost-effective and non-destructive screening method for identifying toxic residues on herbarium sheets; and
- To establish the most suitable decontamination method for the removal of naphthalene from herbarium collections, maintaining the integrity of the specimen.

The research outlines how the presence of fluorescent marks on specimen sheets throughout the herbarium, at Amgueddfa Cymru-National Museum Wales (AC-NMW), is indicative of mercury(II) chloride contamination. Compelling evidence is given to support the hypothesis that the observed fluorescence is due to the reduction of Hg(II) to Hg(I) during the oxidative degradation of cellulose, occurring as part of the natural ageing process. The rate of fluorescence development is shown to be increased by the presence of naphthalene, and is estimated to take at least 30 years to develop.

From the results obtained, it is evident that a hand-held UV-A lamp can provide a rapid and effective method of identifying samples within a collection that are contaminated with mercury, thus providing a rapid and economical means to prioritise which collections require immediate re-mounting. Furthermore, this method will enforce the implementation of safe, standard procedures to protect personnel and visitors when handling the collections, plus enable the removal of a large amount of hazardous chemical from the herbarium environment.

It is also demonstrated, through decontamination tests, that the air-drying of contaminated specimen sheets is a more efficient method of removing naphthalene, than either freeze-drying or oven-drying. It is also the most cost-effective, and the least damaging to the specimen. It is shown that the efficacy of the decontamination is dependent upon the paper type. Thin, unfinished, papers are more efficient at losing naphthalene than the heavier, finished and coated papers, as their porous structure allows the naphthalene to remain more mobile. These results provide important information about which specimen sheets are more likely to be successfully decontaminated by the air-drying procedure and can inform the selection and prioritisation process.

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PART I: INTRODUCTION

1 Historic Overview

1.1 The Origins of Botany and Herbaria

Natural history or natural science is the study of the natural world. Research is carried out through the observations and identification of the natural world and of collections such as specimens of moss, lichens, minerals, insects and birds from the disciplines of botany, geology and zoology.

Plant specimens have been gathered for centuries with the earliest record of plant collecting dating from 1495 BCE (Musgrave, Gardner *et al.*, 1999). Specialists from the 4th century BCE are known to have shown a growing interest in plant science. Aristotle (384-322 BCE) a student of Plato is believed to have questioned the abundant variation in seeds, with specific reference to why certain seeds gave rise to certain plants. His pupil, Theophrastus (372-287 BCE) stated that the study of plants should not only focus on the medicinal properties but should also consider 'their mode of generation and their whole manner of living' (Lazarus, Pardoe *et al.*, 1997). Aristotle travelled with Theophrastus to Lesbos to study the botany and zoology of the island. Theophrastus later produced '*A History of Plants*', where he attempted to identify and describe 450 plants endemic to Greece. Although his descriptions were thought to be lacking in detail; this work was regarded as pioneering within the field of taxonomy and more concrete than that found in Aristotle's writings (Arber, 1912)

Figure 1 Example of 18th century specimen commissioned by the Third Earl of Bute showing original mounting method using wax and straps.

The importance of botany and plant collections (Fig.1) became more than apparent when the Romans, pioneering in all they did, began taking their plants with them once their empire had started to expand. The famous first century herbal[1], believed to be the most important source of plant identification in Europe, was purchased for the Emperor Maximillian II in 1569. This exceptional illustrated copy of '*Dioscorides' De Materia Medica*' was originally published in 512 CE and is now known as '*Codex Vindobonensis*'. It is presently housed in the National Library in Vienna (Arber, 1912, Hobhouse, 1992).

A natural progression towards systematic botany ensued through the need to identify medicinal plants accurately. The ability to discriminate between beneficial or adverse effects of certain species became a central theme of herbals (Arber, 1912). The earliest printed herbals published between the dates of

[1] Herbals are publications concerned with plants that have medicinal properties. Herbals concentrate on the identification of the specimen and how to extract and administer each plant's specific properties.

1470 and 1670, reveal some of our earliest discoveries of botany and apothecaries.

Historically, monasteries became the perfect environment for botanical research to be undertaken. The residents were known to have collected and pressed flowers of interest, later drawing and hand-colouring the specimens to be bound within herbals, providing educational research tools. The essential oils of many plants were also collected and the medicinal properties recorded. Mediaeval monasteries exchanged plants across Europe both introducing new specimens to several countries (Musgrave *et al.*, 1999), a practice still continued today. Gregor Mendel (1822-1884), an Augustinian priest and scientist was known as the "father of modern genetics" for his study of the inheritance of certain traits in pea plants (*Lathyrus* sp.). He was inspired by both his professors at university and his colleagues at the monastery, where he conducted his research.

Over the years, plant hunting became a passion and several pioneering botanists became dedicated to increasing the understanding of botany and horticulture. The first of note was John Tradescant the elder (c.1570-1638) who travelled to Russia and the Low Countries bringing new species to Britain. Together with his son, John the younger, he established the role of the plant collector and stimulated the gardener's passion for creating plant collections. He became a member of the Virginia Company and received many new introductions of North American species (Musgrave *et al.*, 1999). He was head gardener to King James I and also the founder of the first university museum, the Ashmolean Museum, which opened in Oxford in 1683 (Nichols, 2006). Prior to this, his and his son's collections had only ever been displayed in their own home.

Figure 2 Portrait of Linnaeus at 32 by H Sceffel. Oil painting 1739. Reproduction courtesy of Uppsala University Art Collections.

During the 18th century, Carolus Linnaeus (1708-1778), the 'father of botany' (Fig.2), developed an influential system of classification which he published in *Species Plantarum* (1753) and *Systema Naturae* (1758). These became the starting point for modern botanical nomenclature (Rice, 2008). Linnaeus named plants with a generic name followed by a species element, for example, the White deadnettle was named *Lamium album*. The plants that he described had his initial added to the end of the plant's name, for example *Lamium album* L.

Another recognised pioneer was Sir Joseph Banks who accompanied Captain Cook on the Endeavour in 1768-71. Despite terrible circumstances and harsh conditions he circumnavigated the globe and collected 1300 plant specimens which he brought back to Britain (Musgrave *et al.*, 1999, Rice, 2008). The complete series of the botanical plates drawn by Sydney Parkinson are still in existence (Originals: Natural History Museum, London and Prints: AC-NMW, Cardiff) providing an exquisite reference to the numerous exotic plant species that Banks encountered on his expedition.

The mid 19th century gave rise to the Victorian's mania for collecting. Victorians were fascinated with the natural world and their passion for collecting shaped the museum collections and showcases that are still displayed today. Adversely, the passion for collection also meant that numerous species were hunted to extinction, but this did not seem to hinder this newfound lust for knowledge.

Two great names in the theory of evolution and natural selection were Charles Darwin (1809-1882) and Alfred Russell Wallace (1823-1913). The former is probably far better known on these conjectures, but it was in fact Russell's paper that he sent to Darwin in 1858 '*On the tendency of varieties to depart indefinitely from the original type'* that prompted Darwin to publishing *'The Origin of Species by Means of Natural Selection (1859)'*, which changed the way people thought about the natural world and evolution.

Wallace was a British naturalist and explorer, known as the 'father of biogeography'. He was, of note, one of the first prominent scientists to raise concerns over the environmental impact of human activity. He travelled to the Amazon River basin and then to Malaysia and Indonesia and his findings became one of the most popular and influential journals of scientific exploration, published during the 19th century. Unfortunately, he lost the majority of his specimens collected over four years through a fire on board his ship, but some surviving samples are held at the Royal Botanic Gardens, Kew and the Natural History Museum, London. The loss of his specimens is probably the reason that Darwin, who still possessed the actual specimens he collected and could therefore continue his studies with the specimens to hand, overshadowed his influence on evolution.

Darwin and Wallace travelled similar but independent paths. Darwin circumnavigated the globe with a group of surveyors on HMS Beagle from 1831-1836. He collected thousands of specimens that he catalogued and sent ahead of him to various institutions, including the Natural History Museum in London. One such collection consisted of 1529 specimens in spirit, 3907 labelled skins, bones and other dried specimens, as well as a live Galapagos tortoise (Desmond and Moore, 1991). These collections assisted in the development of his theories, which ultimately led to his ground-breaking publications.

1.2 The Herbarium

A herbarium is a collection of dried, pressed botanical specimens with accompanying data labels, usually organised systematically according to a classification system, such as Linnaeus had introduced.

A herbarium is divided into sections: those holding the lower plants (lichens, moss, hepatics, algae, fungi) and those holding the higher, vascular plants (flowering, grass and fern specimens) (Fig. 3). Herbaria exist universally and such collections can be located within 'The Index Herbariorum' which lists c.3000 hrbaria world wide (Holmgren, Holmgren *et al.*, 1990).

Figure 3. Typical example and layout of a NMW herbarium specimen.

Figure 4 A herbarium cabinet showing layout and some higher plant specimens within the collection.

Each specimen is usually mounted onto supporting sheets and labelled (Fig. 4) and placed in systematic order to enable ease of re-classification, research and access to the collections. The herbarium forms a reference library and provides accurate documentation on plant species, habitats, localities and nomenclature. Comparisons of such records have informed scientists of the physiology, morphology, survival, distribution, rise, decline or eradication of a particular species. Herbaria can also include material that has been preserved in spirit, usually industrial methylated spirit and glycerol.

Of particular importance in herbaria is type material. This is the original material from which the description of a new species is made. These specimens are, therefore, scientifically unique, invaluable and irreplaceable and for this reason, many institutions choose to keep their type material separate from the main collections and housed in fireproof cabinets.

Due to their great scientific significance, botanical type material has to be described and this description is published in Latin. All type material should be well documented including details of their history and provenance. Botanical specimens have, through historical practice, been collected, dried, pressed and adhered to a backing sheet. This provides support to the specimen and allows ease of handling, lessening physical damage. The method of adhering over the centuries has varied from gluing, sewing, pinning and strapping. The main substrate has always been paper based, probably because it is readily available and a good method of support. Furthermore, being cellulose based, both the specimen and substrate will react similarly and sensitively together, with both undergoing cellulose degradation over time.

1.3 Preservation Treatments of Herbaria

Botanical specimens consist of organic material containing carbon, hydrogen, oxygen and nitrogen and hence will degrade. Insects, moulds and even rodents attack dead organisms because they are good sources of carbohydrates and proteins and herbaria provide large and easily accessible amounts of nutritious food. Consequently, it has been necessary to protect herbaria from biological attack; if a herbarium specimen or object has not been damaged through biodeterioration in over twenty years, it is probable that the specimen has been chemically treated.

Figure 5 Paper-mounted *Geranium sp.* showing indiscriminate insect damage to flowers, leaves and mount sheet by the biscuit beetle *(Stegobium paniceum)*.

The main insect culprits common to herbaria within the UK are silverfish (*Lepisma saccharina*), booklice (*Liposcelis bostrychophilus*), furniture beetle *(Anobium punctatum)*, biscuit beetle *(Stegobium paniceum*) (Fig.5), tobacco beetle, *(Lasioderma serricorne)* and carpet beetle (*Anthrenus verbasci*) (Pinniger, 1994). The former two beetle species feed on cellulose material and can cause significant damage in a short space of time. The carpet beetle feeds on mainly animal material. Development can be rapid at temperatures c.30°C with generation times as short as five weeks (Pinniger, 1994). Booklice are especially difficult to protect against as they are only 1-2mm in size and are present wherever microscopic moulds are present. The adult lays up to 200 eggs over a period of 6 months and if the conditions are favourable (25°C and 75% RH) the eggs will incubate over 11 days and the insect reaches maturity within just 15 days (Morgan, Price *et al.*, 2006). Booklice feed on microscopic fungi and an infestation can decimate a collection of dried fungi or lichen. Certain plant families are more susceptible to pest attack, especially if they include largely nutritious plants e.g., *Malvaceae, Fabaceae, Capparacea* and *Apiaceae* (Morgan *et al.*, 2006). The *Lactucaceae* (lettuce family) are particularly vulnerable and *Asteraceae* (daisy family), *Ranunculaceae* (buttercup family), or *Brassicaceae* (cabbage family) have flowers rich in pollen, which is high in protein. Fleshy species, such as succulents, are difficult to dry thoroughly and can be prone to both mould and insect attack.

Numerous moulds are ubiquitous within our environment and so protecting material from mould attack is problematical. If the conditions within a storage area are cool and damp, with little or no air changes, then a mould outbreak is most probable. Past methods of dealing with such attacks of both moulds and pests were to apply pesticides and fungicides (biocides). A pesticide is defined as a substance, preparation or organism used to control or destroy any pest. Pesticides can be used to safeguard the public health and protect buildings and other structures from harmful and unwanted pests (HSE, 2009).

Treatments to zoological collections, such as insect, taxidermy and bird skins, have been recorded as far back as the late 18th century and many toxic and hazardous chemicals were applied regularly to bird skins and furs for cleaning, de-greasing and preserving. Arsenic, lead and mercury were found to kill all living things, if the concentration was high enough. Similar applications, although not frequently recorded, were also made to plant specimens. The earliest literature dates back to 1770 (Davis, 1770; Kukahn, 1770).

Natural methods of pest and fungal control were also used and included essential oils of plants such as cinnamon (*Cinnamomum cassia* Blumes), Ajwain (*Trachyspermum ammi* (L.) Sprague), which have been effective as fungicides (Pandey and Srivastava, 1995; Wadhwa & Bairagi, *et al.* 2010) and ginger (*Zingiber cassumunar* Roxb.) and long pepper (*Piper retrofactum* Vahl.) which have proved to be effective insect repellents, although the former specimens have insecticidal properties (Nilvilai and Wangchareontrakul, 1995). Other botanical treatments have included camphor, tobacco, strychnine and perfume of lemon. Dried material was preserved in salts, stuffed with herbs or heat-treated (Goldberg, 1996).

More recent non-chemical methods of pest control have been employed such as freezing the specimens at -20°C for 48-72 hours (Bridson and Forman, 1998), micro-waving (Hall, 1981) and anoxic treatments, where oxygen is removed by flushing with nitrogen or carbon dioxide gas.

Maintaining a stable environment has also been proven to reduce mould and insect attacks that were common in the past. Botanical collections benefit from a stable environment of 20°C ±2°C and 50%RH ±5%. This is too dry for moulds and pest reproduction and the cool temperature is therefore not conducive to pest infestations.

1.3.1 Historic Biocide Applications

Curators were often responsible for developing newer and ever more toxic recipes to protect their collections, reapplying regularly, sometimes on a yearly basis, leading to the accumulation of some of the more stable compounds. The most common applications contained arsenic, lead, mercury, barium and naphthalene. These were sometimes applied singly or as mixtures (see Table 1).

Table 1 Summary of the common preservation recipes used since 1771, giving date of use and preparators.

Preparator	Date	Solvent	Chemicals used
Johann Reihnhold Forster (Forster, 1771)	1771	Water Sal ammoniac (ammonium chloride)	Arseniate of potassa (potassium arsenate) mercuric chloride/arsenic
Edward Donovan (Donovan, 1794)	1794	Alcohol.	Alum (Aluminium sulphate) Arsenic Sulphur Camphor, Tanner's bark, Tobacco
Arsenical soap (Grimes, K. 2007)	1851	Water	White soap (1lb), lime in powder, or chalk (2oz) carbonate of potash (salt of tartar) 6oz, powdered arsenic (1lb), oil of thyme (1oz) Camphor (2 oz).
Wickersheimer's Solution (Kratzer, 1883)	1880	10 litres boiled water, 4 litres glycerine, 0.75 litres wood naphtha	12g arsenious acid, 150g potassium sulphate 60g of common salt, 18g of potassium nitrate, 15g potassium carbonate.
Hough's dry specimen treatment (Hough, 1889)	1889	Powdered chalk/Vaseline	Arsenic
Hough's Global solution (Hough, 1889)	1889	1 pt Saturated Arsenic and alcohol	20 grains Strychnine and 1 pt Naphtha 25 drops strong Carbolic acid (Phenol)
Walter Hough (Hough, 1889)	1889	50% alcohol	Mercuric chloride wash Naphtha crystals
Kew Mixture (Bridson & Forman, 1998)	1917	1 pint methylated spirit	½ oz Mercuric chloride ½ oz Phenol (carbolic acid)
Harold Stuart Thompson	1917		Naphthalene balls
J.W. Franks (Franks, 1965)	1965		Naphthalene balls
J.W. Franks (Fransk, 1965)	1965	1 gallon (4.5 litres) Industrial spirit	4 oz Mercuric chloride 4 oz Phenol (carbolic acid)
Cambridge University Herbarium Mercuric chloride solution	1974	2 litres Methylated spirit	60 g mercuric chloride 60 g Phenol (carbolic acid)
Thomas Croat (Croat, 1978)	1978		Naphthalene balls
Fishmoth poison (Hedges, 2011)	1982	40 parts water	5 pars barium fluorosilicate, 6 parts sugar, 4 parts flour, 5 parts gum arabic

Mercury

Mercuric chloride ($HgCl_2$), synonym corrosive sublimate, was a common and effective pesticide applied to museum and university collections universally. Mercuric chloride has been documented as being used as a pesticide and as a preservative for bird skins from as early as 1770 (Davis, 1770; Kukahn, 1770). It was most widely used on botanical collections because of its fungicidal and pesticidal properties. In 1887, Dr. Walter Hough, head curator of the Anthropology Department at the National Museum of Natural History, Washington D.C., described a variety of methods and mixtures for pest eradication of anthropological items, including recipes of poisonous treatments and suggestions for pest management (Table 1). He was a champion for mercuric chloride stating that:

"It coagulates the albuminoid principles of vegetation and thereby preserves against decay as well as attacks on insects" (Hough, 1889).

Arsenic and Lead

From the 18th century to the late 20th century, arsenic compounds were commonly applied as a preservative to biological specimens and ethnographic objects, not only as insecticides, but also as herbicides and rodenticides (Knapp, 2000). Arsenic compounds had long been used by natural scientists who valued their preservative properties for natural history specimens. In 1940 the former National Museum of Natural History (NMNH), Washington DC, produced a memorandum to document changes in the types of treatments that accompanied the preservation of organic collections. It served as a manual for the treatment of insect infestations and comments from this memorandum suggested that specimens treated with arsenic were very rarely subject to new infestation problems (Goldberg, 1996). Arsenic was also used as a fixative in the preparation of wet specimens and controlled the growth of micro-organisms.

In 1771 Johann Reinhold Forster recommended washing beetles, nuts and seeds with a solution of water, sal ammoniac and arsenic or mercuric chloride, but even earlier records of arsenic use on natural history specimens exist (Hawks and Williams, 1986). Arsenic was applied as a pesticide in several forms, one of the most familiar being arsenic trioxide (As_2O_3), an inorganic trivalent arsenic compound that is an odourless, tasteless, white, amorphous crystalline powder.

The most common of the arsenic based insecticides was lead arsenate ($PbHAsO_4$), a pentavalent form of inorganic arsenic, normally existing as white crystals with no discernible odour. There are two main forms of lead arsenate: acidic and basic. Under most conditions basic lead arsenate is more stable than the acidic lead arsenate, which was used predominantly in Britain (Peryea, 1998). Lead arsenate was first prepared as an insecticide in 1892 in Massachusetts, USA (Peryea, 1998). Its use declined in the 1930s, around the time of the great economic depression, probably due to its high cost and cheaper alternatives such as calcium arsenate and dichlorodiphenyltrichloroethane (DDT) which were being developed as replacements (Peryea, 1998). Lead arsenate was primarily used as an insecticide in museums to prepare organic materials such as furs, leathers and plant material for exhibition. However, taxidermists used lead arsenate as a preservative as well as an insecticide

(Henry, 1996). Lead arsenate contains 22% arsenic (w/w) and is very slightly soluble in cold water (EPA, 1986).

Arsenic and its compounds are known to be highly toxic, capable of attacking the -SH groups present in enzymes and altering their functions (Spalt, Alberti *et al.*, 2005). As early as 1967, the United States Environmental Protection Agency (USEPA) banned arsenic trioxide for home use in concentrations of more than 1.5% and all insecticidal uses of lead arsenate in the USA were officially banned in 1988 (USEPA, 1988). The UK banned its use one year earlier, in 1987. Arsenic compounds retain their toxicity and once treated, objects containing arsenic can probably never be fully decontaminated (Knapp, 2000). Lead is a persistent toxin in the environment and was commonly applied as lead arsenate. Other lead compounds may well have been used on herbarium specimens, although literature on this area is very limited. The Department of Environmental Protection (Maine, USA) states that the only two lead compounds known to be used as pesticides were lead arsenate and lead acetate (Hicks and Montagna, 2009). Due to such recommendations, institutions, such as Cambridge University herbarium, painted plant specimens with ethanol, phenol and mercuric chloride (Briggs, P.D. *et al.*, 1983). Interestingly, by looking through the various types of recipes, phenol (carbolic acid) is frequently added to the mercuric chloride solution. Phenol and mercuric chloride will both cause precipitation of albumen (Potter, 1902). It was common practice in the larger UK institutions, such as the Royal Botanic Gardens, Kew and the Natural History Museum, London, to repeat applications of mercuric chloride, since it was reported to become inert over time (Merrill, 1948, Briggs *et al.*, 1983, Morgan *et al.*, 2006). Its application was continued until as recently as 1982 at the Royal Botanic Gardens, Kew (Clark, 1986). Here the entire specimen was dipped or brushed with a mixture of mercuric chloride, cresol and alcohol. Alcohol is now believed to be a chemical activator of specific mould spores (Florian, 1994) but this relationship was probably not fully understood at the time.

Mercuric chloride treatment was observed to cause darkening and stiffness when used on animal skins, so a recipe of corrosive sublimate in alcohol, with the inclusion of naphtha crystals, was recommended to prevent the re-crystallisation of the mercury salts (Hough, 1889). Historical examples of botanical specimens treated with mercuric chloride have shown grey/black deposits on the sheets (Fig.6). This has been identified as mercuric sulphide (metacinnabar). Mercury reacts strongly with sulphur, either present in the atmosphere (environmental pollution), the paper support, or in animal glues used to attach the specimens or labels to the herbarium sheets. The grey deposits can obscure data and methods have been devised to remove these deposits using chemical applications (Hawks and Bell, 1999). With time (the specimen in Fig.6 is over 200 years old) mercuric chloride becomes mercuric sulphide and is eventually reduced to mercury.

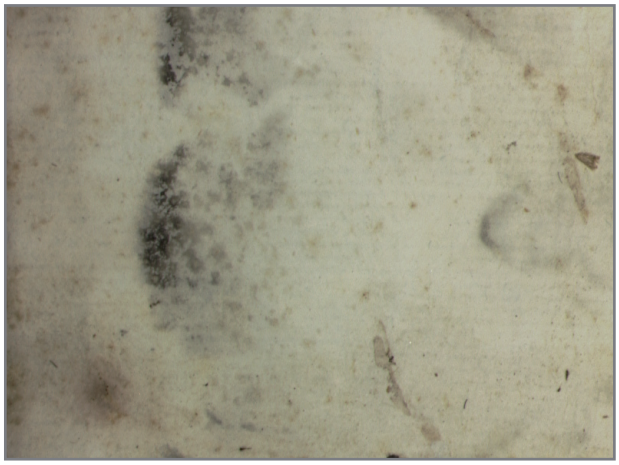

Figure 6 Specimen of herbarium paper from the Third Earl of Bute's herbarium c.1760 showing typical grey discolouration of metacinnabar.

Barium fluorosilicate (barium hexafluorosilicate, BaF_6Si) was patented for use as a pesticide in 1927 by Howard S. McQuaid of the US. Various barium compounds have been used as insecticides and also as rodenticides, for example barium metaborate, barium polysulphide and barium fluorosilicate (Goyer, 1996, Choudhury and Cary, 2001). Herbaria have used barium fluorosilicate and the less effective sodium fluorosilicate, to control silver fish (Bridson and Forman, 1998). Barium fluorosilicate has been reported to be fully effective at preventing silver fish infestation. The barium fluorosilicate was mixed with sugar, starch and glue and was painted into crevices, or on the underside of herbarium cabinets and wooden surfaces. Once applied, it was believed to be permanent (Hawks and Bell, 1999, Morgan *et al.*, 2006) and no damaging effects were observed on herbarium sheets after its use. The paste contained large amounts of sugar to attract the insects and the barium fluorosilicate acts as the poison.

Naphthalene and Paradichlorobenzene (PDB, p-dichlorobenzene, 1,4-dichlorobenzene)

Naphthalene ($C_{10}H_8$) was recommended as an insecticide during the late 19th century (Hough, 1889) insecticides. It is still widely used throughout the United States and large amounts of the chemical are still present in collections, despite the present uncertainty regarding its risks to human health. Naphthalene and PDB ($C_6H_4Cl_2$) were often used in combination and were believed to be extremely efficient at deterring pest infestations.

Naphthalene is supplied in various forms: transparent prismatic plates, white scales, powder balls, pellets or cakes. This makes it very simple to administer to the insides of drawers or to hang in bags within cupboards. Naphthalene is readily available, relatively cheap and its ease of application to the collections has made it one of the most commonly used pesticides in natural history collections in Britain and abroad.

"The sudden introduction of these chemicals into a herbarium that has not been using them will probably be met with vigorous protests from some of the staff. However, it is remarkable how soon one quickly gets used to the smell and eventually fails to notice it…" (Savile, 1962).

This extract was taken from the Canadian Department of Agriculture's Collection and Care of Botanical Specimens (1962) and refers to the introduction of both naphthalene and PDB into the working environment. Naphthalene readily becomes volatile as it has a high vapour pressure of 0.05mmHg (10.9 Pa.) at 20°C and 0.078 mmHg at 25°C (Sonnenfeld, Zoller *et al.*, 1983). It sublimes at room temperature with a characteristic odour of coal tar. With fluctuations in temperature, naphthalene will continue to go in and out of its vapour phase. Even after the removal of the main source of crystals, the strong smell still remains.

Surprisingly, its effectiveness as a pesticide is questionable. Its use in museum collections is prolific the world over, yet it does not kill the pest; it has no effect as a contact pesticide, it has no effect as a fumigant and has been noted as a poor repellent of pests associated with textiles or fabrics (Edwards, 1981). This raises the question to why it has been used for so many years in so many institutions. It is possible that it superseded PDB because PDB sublimes more readily at warmer temperatures. As it was thought to dissipate too readily, it became expensive to replace and so naphthalene was used in its place, as it lasted much longer (Savile, 1962).
Naphthalene is not supported as a pesticide by Commission Regulation (EC) No 1112/2002 [2], as no safe level has been set and the occupational exposure limit has been withdrawn (HSE, 1999).

1.3.2 Methods of Biocide Application

Common applications made by curators and collection managers varied from fumigating the collections, applying chemicals capable of sublimation, dipping the specimens in chemicals, or brushing or spraying the inside of cabinets or mounted sheets.

Fumigation

Fumigation was a common method of treating a large area and therefore a high number of specimens, quickly and cheaply (Fig.7).

One method was to subject the specimens to a continual fume of Zyklon B (cyanide) gas. This was first developed in 1922 and was in wide use as a commercial rodenticide. It was later used in the gas chambers during WWII and is known for its extreme toxicity. Specimens would have been placed within a fumigation chamber and fumed with Zyklon B gas (Fig.8).

2 The Commission is to undertake a programme of work for the gradual examination of active substances that were on the market two years after the date of notification of Directive 91/414/EEC. The first stage of this programme was laid down by Commission Regulation (EEC) No 3600/92 of 11 December 1992 laying down the detailed rules for the implementation of the first stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (3), as last amended by Commission Regulation (EC) No 2266/ 2000 (4).

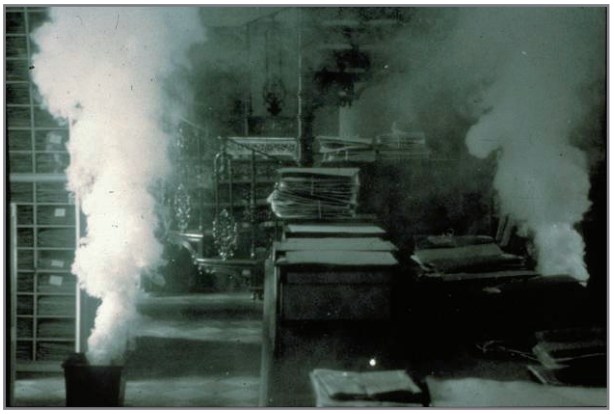

Figure 7 Fuming specimens. Cyanide canisters and pellets were set off in bins to fumigate large areas c. 1960-70s. (Copyright: The Board of Trustees, Royal Botanic Gardens, Kew).

Figure 8 Fumigation chamber. Pellets were released into chambers to fumigate discrete collections. (Copyright: The Board of Trustees, Royal Botanic Gardens, Kew)

The treatment areas were sealed and the collections subjected to this fume for up to 48hrs. The health of staff members, however, was affected by the fumigant-contaminated working environment (Attanatho, Ratanamaneechat *et al.*, 1995) and the highly toxic Zyklon B was probably replaced with other gases deemed to be less hazardous, such as ethylene oxide, phosphine, ethylene dichloride, sulfuryl fluoride and carbon disulfide, sometime after 1945. Methyl bromide (bromomethane, CH_3Br) then replaced these gases, as it was considered less toxic to health and was used effectively on library and archive materials and later, herbarium material right up until 2004, when it was withdrawn under recommendations of the HSC/E as it was considered an ozone depleter (HSE, 2004).

Continuous Applications

Continual applications were applied by exposing the collections to 'off-gassing', volatile or subliming materials (Franks, 1965), such as PDB, naphthalene and dichlorvos ($C_4H_7Cl_2PO_4$). Such methods even included applying camphor, cedar, tobacco and lavender flowers. The material was placed in drawers and cabinets and the smell was believed to deter insect pests. Naphthalene was applied to nylon bags (cut up pairs of tights) in the form of pellets. These were hung on the inside of each cabinet door.
PDB is still in use today, however naphthalene was withdrawn in 1999 pending further medical studies (HSE, 1999) and dichlorvos was withdrawn in 2003 (HSE, 2003).

Aqueous Applications

For aqueous applications, a vat of the biocide was prepared and the specimen dipped and then placed onto the herbarium sheet (Clark, 1986). The solution seeped onto the sheet and when dried, the specimen was mounted. Brushing and spraying were also commonly used application methods.

Mercuric chloride ($HgCl_2$) was a common aqueous application, often mixed with other substances, such as arsenic trioxide (As_2O_3) phenol (carbolic acid, C_6H_6O) (Briggs *et al.*, 1983, Clark, 1986, Hawks and Bell, 1999, Morgan *et al.*, 2006) and lauryl pentachlorophenate (LPCP) (Womersley, 1981, Bridson and Forman, 1998, Morgan *et al.*, 2006).

A mixture of mercuric chloride and phenol in aqueous solution was often painted directly onto the herbarium specimen (Briggs *et al.*, 1983) (Fig.9). The phenol was sometimes replaced with 3-methyl phenol (cresol, $CH_3C_6H_4OH$), as this was thought to be an effective fungicide (Morgan *et al.*, 2006).

Figure 9 Application of Kew Mixture to herbarium specimens' c. 1970. (Copyright: The Board of Trustees, Royal Botanic Gardens, Kew).

Paste Applications

A paste application was not so common a method. The paste was not applied to the specimen itself, nor the sheet, but to the back, or underside, of the cabinet or wooden surfaces near to where the specimen was to be housed. Once applied, it was believed to be permanent (Morgan *et al.*, 2006). Barium fluorosilicate or sodium fluorosilicate were used, mixed with a bait of sugar and starch (Morgan *et al.*, 2006). No damaging effects were observed on herbarium sheets after its use.

1.4 Problems Associated with Historic Treatments

Historically, herbarium preservation methods and treatment histories have been recorded but not always to the extent, or with as much detail, as zoological preparations and therefore the level of contamination within herbaria collections is broadly unknown. This lack of data not only prevents the amount and type of contamination being disclosed from a health and safety perspective, but also has implications when undertaking intrusive molecular research on the organic material, as DNA extraction and amplification can be hindered by pesticide treatments. Certain chemicals, such as mercuric chloride, have been suspected of hindering molecular studies (Bridson and Forman, 1998). Methyl bromide, methyl iodide, ethylene oxide, propylene oxide, chromates and some arsenic compounds affect both extraction and amplification of DNA (Hall, 1997, Kigawa, Nochide *et al.*, 2003). Arsenic was understood to affect DNA extraction and it has since been confirmed to inhibit polymerase chain reaction (PCR) (Topfer and Haring, 2009). This could negate the usefulness of specimens being kept within a reference collection. If it is intended for material to be used for this

purpose, it would be beneficial to know which chemicals have been applied in the past and whether further research, including chromosome or DNA extraction, is possible.

Biocides were applied to herbaria because of their toxicity to pests and moulds. It is unlikely that many of the natural history specimens or ethnographic materials of organic origin would have survived the last three hundred years without the aid of these biocides. Many of the historical biocides were, however stable and residues are still present today, posing a hazard to personnel working within the collections. Furthermore, the contamination is unlikely to be restricted to the specimen itself. Due to the morphology of most botanical specimens, their waxy cuticles would have reduced, if not prevented, absorption of the biocide. It is the herbarium sheet that would have absorbed the majority of the chemical. Handling these sheets today could lead to contamination through skin absorption and inhalation. Unfortunately, due to the poor recording of historic applications, there is little data relating to past treatments made to the specimens.

Identifying past biocide treatments is, therefore, difficult. It may be possible to determine the probable treatments applied through existing (although scant) documentation, communication with past curators or senior staff members and through a process of elimination. Several factors can be taken into consideration with botanical material: the date the specimen was collected; the country of origin of the specimen, standard practice of that period and most importantly, the pest that the collector/curator was attempting to eliminate.

2 Paper Structure, Composition and Degradation

2.1 Cellulose

Over the years, paper has been made from many plant materials such as cotton, flax, hemp, straw and wood; all cellulose-containing materials. The pulp is macerated and washed in water, which allows the cellulose fibres to separate and form a suspension. A sieved mould is used to pick up the fibres and the excess water drains away leaving a fine deposit of wet pulp, which will dry forming a recognisable sheet of paper (Williams, Fowler *et al.*, 1977, May and Jones, 2006). Paper is a complex material lacking homogeneity on both a macro and microscopic level. It contains several different components in addition to the cellulose fibres, including image media (May and Jones 2006), making it a very challenging matrix to study.

Cellulose is a linear homo polysaccharide composed of β-D-glucopyranoside units, linked by (1→4) bonds (Kontturi, 2005) (Fig.10). The repeating units, or monomers, (given in brackets) repeat themselves many times (represented by n) to form long chains. The monomer is called anhydroglucose and the characteristics associated with cellulose are observed when $n \leq 30$ (Kontturi, 2005).

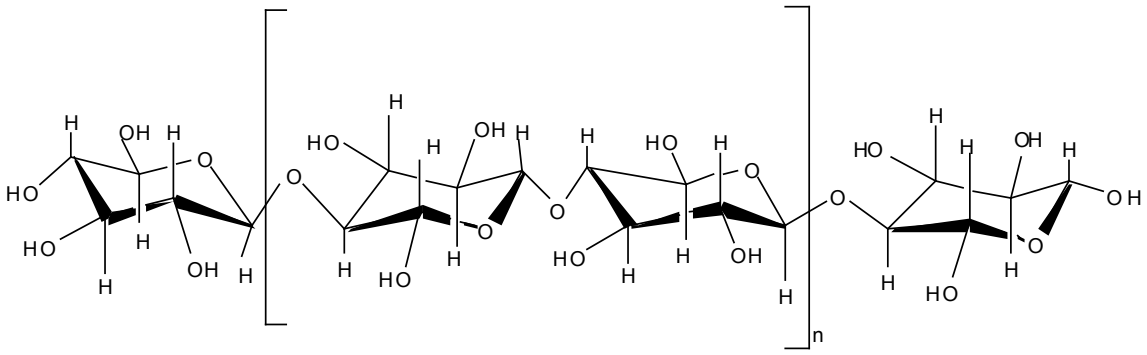

Figure 10 The basic structure of cellulose

There are four crystalline structures of cellulose, given as types I, II, III and IV. Only type I occurs in nature (Tímár-Balázsy and Eastop, 1998). Cellulose I can be transformed to cellulose II by strong alkaline solutions (10-22% potassium hydroxide) causing mercerisation, which means that the cellulose is chemically unaltered but is physically different, mainly through dehydration (Tímár-Balázsy and Eastop, 1998). Also cellulose II can be formed by precipitation of dissolved cellulose known as regeneration (Kontturi, 2005). Cellulose I is very stable and occurs in nature only with other materials such as lignin, beta and gamma cellulose, hemicelluloses and impurities, such as resins, waxes and tannins. Cellulose I is further distinguished by two allomorphs: cellulose I_α and I_β. Cellulose I_β is dominant in bacterial and algal cellulose and cellulose I_β in higher plants such as cotton and wood.

The chains of cellulose are held together by hydrogen (H) bonds which connect the cellulose chains together, forming a supramolecular structure (Fig.11). The H-bonding affects the structure intramolecularly, giving rise to ordered, crystalline regions and disorganised, amorphous regions.

This gives the characteristics of both the stiff and flexible properties of cellulose (Tímár-Balázsy and Eastop, 1998).

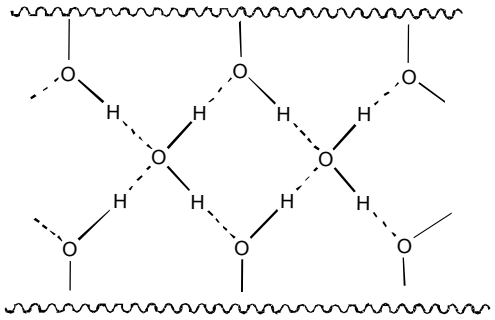

Figure 11 A single layer of water molecules showing the hydrogen bonding (dotted lines) between cellulose chains within the amorphous region.

In Medieval Europe, circa 1200 CE, the first papers were produced from pure cotton and macerated rags (May and Jones 2006). This made very stable paper, as cellulose derived from cotton has few impurities compared to more recently used materials such as straw and wood pulp. The stability of cellulose varies depending on its source (e.g. cotton and wood differ greatly). A sensitive method for testing the differences in the quality of cellulose is through measuring the degree of polymerisation (DP). The average number of monomer (anhydroglucose) units is commonly used to relate to the molecular weight (May and Jones 2006). The strength of a fibre is dependent upon the length of the cellulose chain, therefore high polymerisation relates to high chain length. The chain length can be affected by many different variables, including impurities, external pollutants and addition of chemicals. Cotton and linen have naturally high DP and therefore have an added advantage over wood fibres after the extraction process, where the DP is much reduced (May and Jones, 2006).

2.2 Degradation of Cellulose

The degradation of cellulose in paper is evident through discolouration, drop in pH, a musty, acrid odour, embrittlement, reduced flexibility and brightness (Buelow, Begin *et al.*, 2000).

This process is also visible under UV light at a region described as the wet/dry interface. When moisture is added to paper, for example, it is thought that degradation products are washed outwards, forming a fine tide-line at the wet/dry interface. At this point, these products are oxidised forming a fine brown line in visible light and a bright blue fluorescence under UV light (Pedersoli and ligterink, 2001).

The processes of cellulose breakdown; the products formed and the influence of external parameters, is a widely published area (Iversen, 1989, Ligterink, Porck *et al.*, 1991, Havermans, 1995a, Havermans, 1995b, Dupont, 1996a, Baranski, Dziembaj *et al.*, 2000). There are believed to be five main contributing factors to cellulose degradation:

- acid catalysed hydrolysis;
- metal catalysed oxidation;
- oxidation from light and temperature;
- effects of moisture; and
- mould.

2.2.1 Acid-catalysed Hydrolysis

W. J. Barrow was the first person to make the link between acidity of paper and its permanence (Barrow, 1959). The acidity within the paper is caused by acid-catalysed hydrolysis of the cellulose chains. An excess of hydrogen ions will attack the oxygen bridge between monomers and break the bond, forming a hydroxyl group on the broken bridge. This process continues until the H^+ ions are removed, effectively lowering the acidity. Additionally, the process leads to a reduction in chain length, which affects the paper stability (Bansa, 2002).

Paper can start life inherently acidic, due to its method of production and the addition of acidic products, such as sulphites and alum rosin, or through external pollutants, such as nitrous oxides and sulphur dioxide:

$$SO_2 + H_2O \rightarrow H_2SO_3$$
$$NO_2 + H_2O \rightarrow HNO_3$$

The addition of chemicals (for example the use of acid producing chlorine and bisulphites in the removal of lignin and impurities) is one of the main causes of the accelerated degradation of paper (APPA, 1965).

It is perceived that there are two main products of cellulose degradation: hydrogen peroxide (Cunha, 1987, Dupont, 1996a, Dupont, 1996b, Svensson and Alwarsdotter, 1998, Bansa, 2002); and acids (including sulphuric acid) (Cunha, 1987, Bégin, Deschatelets *et al.*, 1999, Bansa, 2002). However, peroxides are formed in the presence of metal ions and not directly through the cellulose degradation process.

Pollutants can adsorb on to the paper and react with the residual moisture within the paper, forming acidic products. These products attack the glycosidic linkages which are very susceptible to damage in acidic conditions.

The processes associated with acid-catalysed degradation of cellulose are summarised in Figure 12.

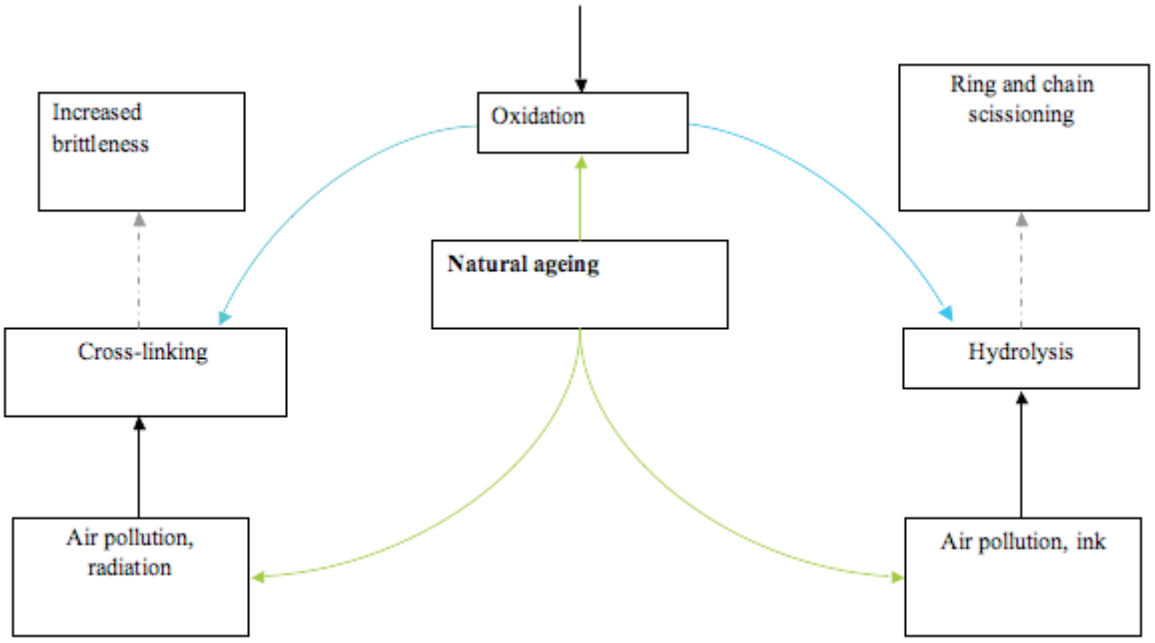

Figure 12 Cellulose degradation associated with acid-catalysed hydrolysis caused by internal and external factors (reproduced courtesy of Dr J.B.G.A Havermans PhD thesis (Havermans, 1995b).

Neevel (Neevel, 1995) suggested that the deterioration of paper is caused by two separate processes: acid-catalysed hydrolysis and metal-catalysed oxidation, which under certain conditions can proceed independently, even when the other process is arrested. It is believed that the degradation products of one process are the initiators for the other (Iversen, 1989).

2.2.2 Oxidation: Catalysed by Transition Metals

During industrial oxidative bleaching of pulp paper, the presence of traces of iron and copper can cause extensive damage to the cellulose.

$$Cu^{+} \rightleftharpoons Cu^{++} + e^{-}$$

$$Fe^{+++} + e^{-} \rightleftharpoons Fe^{++}$$

An example of the initiation of a chain reaction is:

$$Cu^{++} + ROH \longrightarrow Cu^{+} + RO^{\cdot} + H^{+}$$

These reactions are known as the Fenton reaction and are caused by the presence of metal ions from copper acetate pigments, metal ion generated foxing and iron gall ink.

The Fenton reaction was used as one of the first hydroxyl radical decomposition methods of biocides. Iron complexes and hydrogen peroxide, with the influence of UV irradiation, have been attributed to the breakdown of biocides such as atrazine (Bourke, 1992):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-}$$

$$HO^{\cdot} + RH_2 \rightarrow HR^{\cdot} + H_2O$$

The degradation of paper, generated by the presence of transition metals, can be so severe that the area covered by the colorant falls out of the sheet and the area is lost (May and Jones., 2006). Transition metal ions are powerful oxidation catalysts; during a valency exchange, electrons are either donated or accepted, causing reduced chain length of the cellulose (Weinstock, Barbuzzi *et al.*, 2001). Oxidation of a metal fragment can often be seen as a spot with a distinct dark centre and a paler surrounding discoloration of the soluble degradation products (Liu, Lei *et al.*, 2003, Zheng and Yao, 2006). This is often classed as foxing, which relates to a brown stain on paper (often attributed to moulds).

2.2.3 Oxidation: Light and Temperature

Both light and temperature are responsible for initiating oxidation in paper. Direct photolysis does not occur under UV light (340nm) but photo-sensitized degradation does begin where the energy absorbed by photo-sensitizers is transferred to initiators, resulting in the formation of a reactive species. This is the main source of light-induced decay of cellulose and it is thought that a comparable free radical mechanism is responsible for both photo-induced and thermal degradation (Malešic, Kolar *et al.*, 2005). Hydrogen peroxide is thought to be the key chemical in the initiation of polymer photo-oxidation as it is a very strong oxidant (Svensson and Alwarsdotter, 1998, Bansa, 2002).

Temperature is also a significant contributing factor to cellulose degradation. A rise in temperature of 10°C will double all chemical reactions occurring (Wurster, 1888). This is why temperature is used to provide controlled ageing of paper for research. Given sufficient activation energy, hydroxyl groups can cross link via condensation reactions. Highly reactive aldehyde and keto groups are formed with a rise in temperature and are also prone to cross-linking (Bansa, 2002). This results in loss of water and inevitable embrittlement of the material.

2.2.4 Effect of Moisture

During paper processing, moisture is often incorporated into the material, whether intentionally, or unintentionally, through condensation. The uneven distribution of moisture through the paper leads to the development of moisture spots, which can be observed immediately after formation under UV light. It appears as bright blue fluorescence and, over time, becomes observable in visible light as brown stains. The process of gaining water through hydrolysis of the cellulose polymer is known as condensation and this can release hydrogen peroxide as the chains become dehydrated. Condensation can cause physico-chemical changes in paper and areas of uneven moisture are more degraded and acidic than the unaffected areas. This often causes discoloration, including the common effect known

as foxing (Zheng and Yao, 2006).

If moisture is present within an acidic environment, the hydrogen ions will attack the bonds, breaking the chains, thus reducing the fibre length and paper strength (Beazley, 1991, Bansa, 2002).

2.2.5 Effect of Mould

Fungal damage is apparent in visible light as a brown stain, varying in intensity. These stains are seen to fluoresce under UV light (Florian, 1994). However, both mould and paper degradation products have been seen to fluoresce (Pedersoli Jr. and Ligterink, 2001, Florian, 2002). It is possible that oxidation at the paper surface, within the wet/dry interface, causes the cellulose to break down sufficiently to form simple sugars, thus providing the mould with easier access to the paper and its food supply (Arai, 1995).

Micro-organisms ubiquitous within the environment thrive on organic material such as paper under certain environmental conditions: 75-85% RH and 20-30°C. This can promote the most common chemical reaction, hydrolysis, which is accelerated in humid conditions in the presence of the enzyme hydrolase. Hydrolysis of the cellulose is accompanied by oxidation, since bacteria and micro-organisms produce hydrogen peroxide during the decomposing process and the acidic products of metabolism excreted by certain micro-organisms (e.g. one mould in particular, Aspergillus niger, produces citric acid after feeding on sugars) can cause acid hydrolysis of cellulose (Tímár-Balázsy and Eastop, 1998). This too will initiate the Fenton reaction (see Fig.13). Under such conditions, conidia and ascospores germinate and form colonies by feeding on the degraded cellulose, utilising malic and citric acid that the growing hyphae metabolise. The hyphae also deposit 16 amino acids into the colony, which are believed to be responsible for foxing (Arai, 1995).

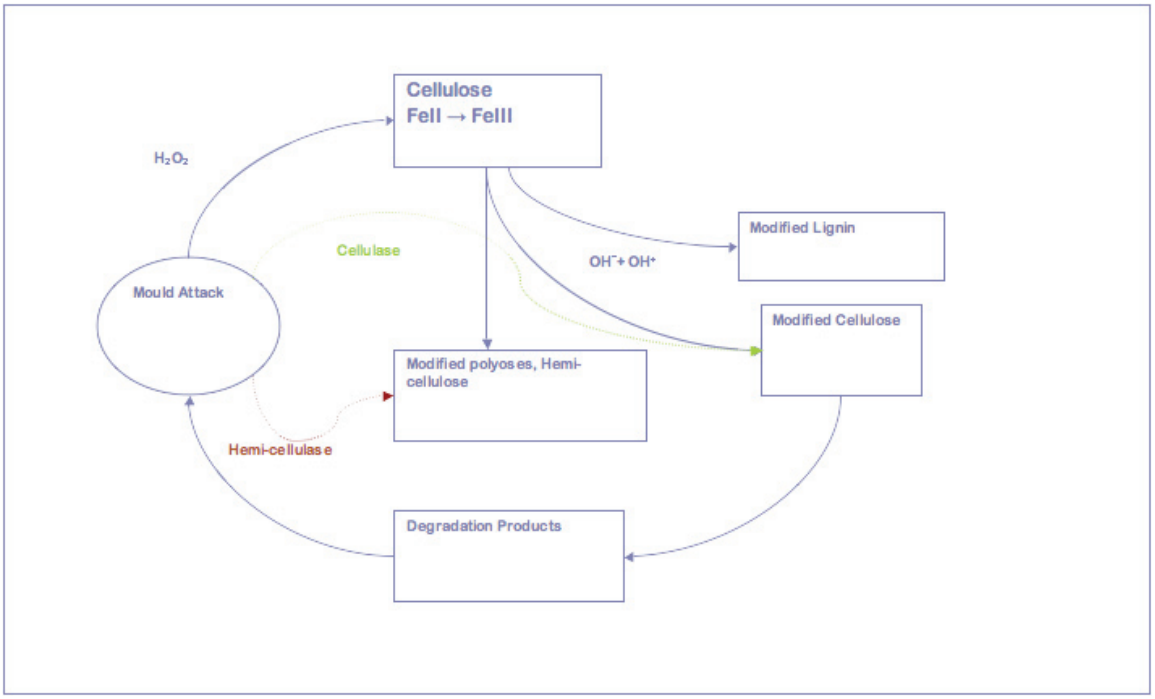

Figure 13 Hypothetical degradation mechanisms of brown-rot fungi (Ritschkoff and Mahlberg, 2001) modified by J. Havermans (Havermans, Aziz *et al.*, 2007).

2.3 Additives in the Manufacture of Paper

Paper is formed from an emulsion of fibres derived from materials such as linen, cotton, hemp, straw and wood. The process of breaking down fibres to form a pulp to make paper is believed to have been first conducted in China in 200 BCE, when the first moulded paper made from hemp fibre was found (Collings and Milner, 1992). The methodology was patented in China by Ts'ai Lun 300 years later and the method perfected by Tso Tzu-yi in 150 CE (Hunter, 1978). By 450 CE the Chinese papers contained mixtures of hemp, mulberry and ramie with starch sizing and a gypsum surface treatment. It is possible that aluminium sulphate was also used around this period and would have been used to harden the gelatine or glue size (Collings and Milner, 1992). In 674 CE it became compulsory to treat Chinese papers with toxic plant juices from the berry of the Amur Cork Tree (*Phellodendron amurense*). This coloured the paper yellow, but also protected the paper from insect attack (Hunter, 1978). Records indicate that cotton was first introduced into British papermaking in 1049, but it was not until three hundred years later, in 1342, that the first paper comprising linen fibres was found (Collings and Milner, 1992).

Additives, including chemicals and minerals, have been incorporated into paper both intentionally, for improving the surface, structure or colour of the paper and unintentionally, through washing, beating and rinsing procedures. The use of fillers or loadings to bulk out the fibres became necessary in 1733 due to shortages in raw materials. China clay was used to plump up the fibres and to increase the weight of the paper, thereby increasing the amount of paper made by weight, but at a cheaper cost. As global demand for paper grew, cheaper paper was produced by replacing expensive and exhaustive supplies of rags, a good source of cotton fibres, with cheaper, but inferior materials such as wood pulp and straw. In order to achieve a comparable standard to cotton-rich paper, however, chemical additives were required to improve the structure, printing characteristics and appearance of this cheaper paper.

Fillers were used to enhance brightness, opacity and smoothness of the paper surface and coating pigments were used to refine the paper surface in preparation for imaging. The minerals used as coating pigments were finer and brighter than those used as fillers and were held within a binding layer such as casein, starch or aqueous emulsions of synthetic polymers. Chemicals have also been used to improve the fibre strength, to colour and to optically brighten the papers (Burns and Potje, 1990).

Sizes were used as far back as 450 CE, but animal size such as gelatine was not used in Europe until 1337 (Hunter, 1978). Sizing treats the fibres making them impervious to writing inks and many different sizes were used to smooth the paper surface and improve the quality of writing. In 1807 Moritz Friedrich Illig publicised his use of alum-rosin sizing in Germany, which due to its high acidity, became the greatest cause of paper deterioration from that point on (Barrow, 1967).

In 1820 Hansard and Murray reported the use of calcium and barium sulphates as fillers, which

were later condemned as contaminants (Collings and Milner, 1992). Subsequent fillers included zinc oxide, zinc sulphide and titanium dioxide, which although much more expensive, produced a finer finish (Grant, 1937). The introduction of these fillers incorporated deleterious chemicals into the paper which have affected its longevity, particularly sulphurous products (APPA, 1965). Sulphur has been incorporated via fillers and the chemical pulping process, which first used sodium sulphate and later, the sulphite process, where bisulphites and sulphurous acid were used to improve the pulp (Rydholm, 1985). Damage to leather bindings and the presence of dark grey mercuric sulphide marks on herbarium sheets have been attributed to air-borne pollutants such as sulphur dioxide, but it is apparent that sulphur was probably already present within many of the papers.

Since the water used for these procedures would vary depending on the geology of the local area, different geographic locations give rise to variation in type and concentration of minerals (and hence metal ions) within the papers.

Table 2 summarises the various sources of metal ions within the paper-making process. Such evidence has been effective in establishing provenance of stone and ceramics and it is therefore likely that the composition of paper will provide information relating to its provenance. Timothy Barrett, for instance, carried out extensive research on historical papers made between the years 1400-1800. Taking into account the different paper-making methods and different sources at the mills, he found the most common elements present within these papers were magnesium, aluminium, sulphur, potassium, chlorine, silica, calcium, manganese, iron, copper, zinc and lead (Barrett, 1989).

Table 2 Metal ions incorporated into paper through the paper-making process

Source	Components	Metal ions
Soils [1]	Clay, limestone	Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}
Igneous Rock [2]	Pyroxene, biotite	$Si^{4+}Na^{+}$,K^{+}, Mg^{2+},Fe^{2+},Fe^{3+},Al^{3+}, Ca^{2+}
Sedimentary Rock [2] Sandstone, Limestone	Feldspar, quartz, calcite (calcium carbonate), magnesium carbonate, gypsum (calcium sulphate, $CaSO_4 2H_2O$)	Ca^{2+}, Al^{3+}, K^{+}, Mg^{2+}, Na^{+}, Si^{4+}
Metamorphic Rock [2] Granite, Slate, Marble	Feldspar, mica, hornblende, quartz (SiO_2) calcite ($CaCO_3$), kaolin hematite (FeO_3) magnetite (Fe_3O_4)	Ca^{2+}, Al^{3+}, K^{+}, Si^{4+}, Mg^{2+},Fe^{2+},Fe^{3+}, Na^{+}
Agricultural and contaminated run off [2]	Animal manure, fertilisers, mining waste, flood waters	Cu^{2+}, Zn^{2+}, As^{3+}, Ca^{2+}, Al^{3+}, K^{+}, Si^{4+} Mg^{2+}, Fe^{2+},Fe^{3+} Se^{4+} U^{6+}, Ni^{2+}, Pb^{2+},Cd^{2+}, Sr^{2+}
Wood [3]	Various pulped woods	Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Co^{3+}, Ba^{2+}.
Size [4]	Alum rosin size	Al^{3+}
Fillers [5]	China clay, barium sulphate, calcined clay kaolin $Al_2Si_2O_5(OH)_4$, titanium dioxide, ultra fine fillers.	Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Co^{3+}, Ba^{2+}, Ti^{2+}, K^{+}, Na^{+} Si^{4+}
Coatings [5]	Calcium carbonate, magnesium carbonate, china clay, kaolin, titanium dioxide, talc (magnesium silicate hydroxide)	Ti^{2+}, Mg^{2+}, Pb^{2+}, Ca^{2+}, Al^{3+}, Si^{4+}, Fe^{2+}
Coating Pigments [5]	Blanc fixe (precipitated barium sulphate), satin white (calcium aluminium sulphate)	Ba^{2+}, Ca^{2+}, Al^{3+}
Pigments [6, 7]	lead chromate ($PbCrO_4$), lead white ($PbCO_3.Pb(OH)_2$ red lead (Pb_3O_4), vermillion (HgS), orpiment (As_2S_3) lemon yellow ($BaCrO_4$), Venice, Hamburg and Dutch whites ($BaSO_4$)	Pb^{2+}, Hg^{2+}, As^{3+}, Ba^{2+}
Deacidification [4, 8]	Zinc oxide, magnesium carbonate, magnesium bicarbonate, calcium hydroxide, calcium bicarbonate	Zn^{2+}, Mg^{2+}, Ca^{2+}, Ca^{2+}

1 Winkler, E. M. (1973) Stone: Properties, Durability in Man's Environment, Springer-Verlag, Vienna.
2 Seaman, J. C., White, N., Kingery, W. and Karathanasis, T. S. (2005) In Multi-State Research Project Proposal (Devel opment Committee) Statement of Issues and Justification. Identifier Number DC201.October 1, 2003 to September 30, 2008, Vol. 2007 University of Georgia, Georgia.
3 McCrady, E. (1996) Alkaline Paper Advocate, 9.
4 Collings T & Milner D (1992). A new chronology of papermaking technology. Institute of paper conservation 14:58-61
5 Beazley K. (1991). Mineral Fillers in Paper. The Paper Conservator 15: 17-27.
6 Harley, R. D. (1982) Artists Pigments c. 1600-1835: A study in English Documentary Sources, Butterworth Scientific, Frome.
7 Burgio, L., Clark, R. J. H. and Rosser-Owen, M. (2007) Journal of Archaeological Science, 34, 756-762.
8 Hey, M. (1979) The Paper Conservator, 4, 66-80.

Lindsay Spence *et al.* have looked at the elemental compositions of different papers by using inductively coupled plasma mass spectrometry (ICP-MS). Their research, prompted by the need for forensic evidence to discover forgeries of papers and manuscripts (e.g. added counterfeit pages to last will and testaments), identified nine main elements: sodium, magnesium, aluminium, manganese, strontium, yttrium, barium, lanthanum and cerium (Spence, Baker *et al.*, 2000). Further research also successfully differentiated papers sent as threatening letters to the business partner of a murdered man from papers seized from the murder suspect, thus introducing a further suspect. This is the first time that paper provenance and its elemental identification has been used to help in a criminal investigation (Spence *et al.*, 2000). The elemental results of the two studies vary largely, indicating that paper will vary both chemically and elementally, but also that paper analysis can provide information on its provenance.

The chronology in which additives were introduced to the paper-making process is summarised in Table 3.

Table 3 Chronology of the paper-making process showing metal ion incorporation

Year	Metal Ion	Processes	Product
450	Calcium[1]	Surface treatment	Calcium sulphate
450	Aluminium [2]	Size	Aluminium sulphate (Alum rosin)
8th C	Magnesium [3] Calcium [2]	Filler,	Talc, chalk, gypsum, calcium sulphate, magnesium silicate
9th C	Aluminium [1, 4] Magnesium [3]	Filler	Talc
1733	Aluminium, Iron, Titanium, Calcium, Magnesium, Potassium, Sodium [1,5]	Filler	China clay
1792	Calcium [1]	Sulphite Process Bleaching	Calcium hypochlorite
18th C	Magnesium [3] Calcium [3]	Filler	Gypsum, talc, plaster of Paris (Calcium sulphate hemihydrate)
1820	Barium [3, 7]	Filler	Barium sulphate
1823	Calcium [3,6]	Filler	Gypsum (calcium sulphate)
1851	Sodium [7]	Washing	Caustic soda Sodium hydroxide
1870	Aluminium [3] Silica [3]	Filler	China clay (kaolin) $Al_2Si_2O_5(OH)_4$
1874	Magnesium [3]	Absorbent and surface treatment	Talc, magnesium silicate
1877	Sodium, Zinc [7]	Bleaching	Sodium/zinc hydrosulphite
1890	Lead [7]	Coatings	White lead, lead carbonate
1925	Calcium [7]	Filler	Calcium carbonate
1927	Calcium [2]	Coating, pigment, filler	Precipitated calcium carbonate
1928	Titanium [7]	Filler	Titanium dioxide
1935	Zinc [7]	Filler	Zinc sulphide
1960	Magnesium [2, 3] Silica [2], Calcium [2]	Filler	Talc, chalk

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3 The National Museum of Wales Herbarium (NMW)

3.1 A Brief History

Figure 14 Amgueddfa Cymru-National Museum, Wales. The NMW herbarium is situated on the right hand side of the building.

Amgueddfa Cymru-National Museum Wales (AC-NMW) (Fig.14) has held a herbarium, the National Museum of Wales Herbarium (NMW), since 1907 when the museum was founded. The Royal Charter of Incorporation was granted in that year by King Edward VII. The first building stone for the present build was laid by King George V in 1912 and then finally, after the war ended, the building was finished and the entire museum collection moved to its present location within the Civic Centre of Cardiff in 1927.

The herbarium is now housed within the Department of Biodiversity and Systematic Biology and holds c. 561,000 specimens, of which 260,000 are vascular specimens. The collection contains specimens dating as far back as the 17th century, but it is possible that even more historical records exist. It is a collection that is thoroughly researched both in-house and through external loans. Due to its age and number of specimens, it has become a priority to determine the status of this collection from a health and safety perspective for staff, visitors, volunteers and researchers

3.2 The Herbarium Sheets

The NMW herbarium collections have been acquired from various different countries and institutions. The specimens are mounted on varying colour, size, weight and quality of paper, none of which are uniform (Fig.15). The type of paper is often chosen by the collector to serve a purpose, but all are cellulose-based, whether from cotton, flax, wood or even straw. The sheets may be of a certain size to fit a personal collection or of a specific weight and strength to support large and bulky specimens. This has given rise to non-standardisation within the herbarium. Some of the specimen sheets are of an unsuitable size for the NMW cabinets; or lack support or simply are inappropriate from a

conservation standpoint. For example, some specimens have been glued onto sheets, as opposed to the preferred method of strapping, which is reversible. It is a policy of the NMW herbarium to re-mount old material. This aids standardisation of the material, improving the conservation standard of the specimen and improving the quality of data, as the majority of information has changed over the years and can therefore be updated and made more relevant to the present day.

Figure 15 Ex British Herbarium sp. sheet, dated c. 1900 showing acid migration from specimen to paper. Paper very pale cream and fine c. 50 gm^{-2}

Each botanical specimen housed within the NMW has been mounted either in-house, by the botany staff, or by external collectors or curators. Each sample comprises of; the specimen mounted onto a mount sheet and adhered to the sheet either by glue or linen strips, a data label relating to the name, date and locality of where the specimen was found and by whom (See Figs.1,3 and 4 for examples of mounted specimens).

3.3 The Effects of Cellulose Degradation within the Herbarium

Taking into account the numerous parameters that can affect the degradation of cellulose, it is apparent that the NMW herbarium material will be undergoing some degree of cellulose degradation. The majority of herbarium sheets date from the 1900s and so have been naturally ageing for many decades. The NMW herbarium has large south facing windows and, although the collections are

housed in cupboards, material is often brought into the light for examination and occasionally for display purposes. It is therefore probable that photo-degradation will be an influence on the collection, the extent of which is dependent upon the total light exposure. The herbarium is not air-conditioned and therefore the collections are at risk from environmental pollutants, including sulphur dioxide and contaminants applied to the specimens as biocides. The fluctuating RH and temperature will also affect the material, as these fluctuations are frequently greater than ±5% RH and ±2°C temperature.

Under UV light, spots are visible on the herbarium sheets within the collection, particularly around the area where the plant was placed. These spots fluoresce over a range of colours, but only within aqueous tide marks or splashes, indicative of an aqueous application.

The literature suggests several causes for coloured fluorescence on paper. Reißland *et al* state that iron gall ink shows a characteristic green fluorescence after accelerated ageing under UV light at λ365nm (Penders and Havermans, 2002, Reißland, 2002). It is also proposed that this visible progress of paper degradation by iron gall ink is comparable to phenomenon such as foxing and of that observed at the wet/dry interface (Reißland, 2002). Tang advocates that the presence of mould can lead to a mould-produced colour that is observed under UV light (Tang, 1978), whilst Beckwith *et al* suggest that it is most probable that the colour change caused by foxing almost invariably indicates degradation of the paper at that specific point (Beckwith, Swanson *et al.*, 1940).

Oxidation of cellulose is often observed in paper as a brown discolouration, due to the formation of unsaturated compounds. This occurs after the initial stages of degradation and usually contains carbonyl groups with conjugated double bonds (Dupont, 1996a, Zheng and Yao, 2006). It is these conjugated bonds that give rise to the colouration and probably the fluorescence often observed with degraded cellulose. Carbonyl groups are recognised chromophores that shift the absorbance of oxidised cellulose from the near ultra violet to the near visible. On further oxidation the carbonyls form carboxyls which do not show colour, but lead to fading in the final stages of photodegradation (Tímár-Balázsy and Eastop, 1998).

From the literature, it is evident that fluorescence on degraded/aged paper is commonplace and generally accepted as being due to cellulose degradation and foxing. However, the colours observed on the herbarium sheets at the NMW vary greatly between grey, white, cream, yellow, peach and orange. Not all of these colours are observed through paper degradation; white/blue is observed at the wet/dry interface and peach from foxing. Green may be observed from iron gall ink.

Although metal-catalysed oxidation will affect the cellulose, the more typical ions such as copper from pigments and iron from iron gall ink, will be largely absent from the herbarium sheets and not the likely cause of the fluorescence. Other elements or compounds, however, may be present in the paper, whether from the paper-making process, or from applications of biocides.

3.4 Pesticide Use within the NMW Herbarium

The only confirmed pesticide treatment adopted by the NMW was the placing of naphthalene pellets to the insides of the specimen cupboards during the 1970s (Tipper, 2001) (Fig.16). These were removed in the 1980s, although by that time the naphthalene had permeated the wooden cabinets and the sheets present within, leaving a pungent aroma.

In 1988, after a break out of booklice (*Psocid* sp.), the cabinets were emptied of specimens and sprayed with Constrain®, a permethrin-based pesticide. Drione, a pyrethrum-based powder, was scattered along the edges of the shelves. Today, only Constrain® is used in the cabinets, as Drione has been withdrawn from use.

Figure 16 Typical old NMW herbarium cabinets. Small brown bags on inside of cabinet drawers used to contain naphthalene pellets which were removed in mid 1980s.

From the published literature and communication with senior staff, no reference has been made to the application of aqueous chemicals directly to any NMW herbarium specimen since the opening of the museum's new build in 1927. Previous to this date, there is reference to one collection having been treated with mercuric chloride in 1909 and 1917, several years after its acquisition by the museum. Unfortunately, this date precedes all existing records and present staff memories and remains unconfirmed.

It is assumed that, at some point in the past, the original collector, or institution, has applied historic pesticide treatments to the NMW specimens. All collections are therefore presumed to be treated and hence hazardous. The current policy to remount old specimens will therefore have long-term benefits in removing the majority of presumed contamination from a sheet. This could, however, expose a member of staff to an unidentified and unspecified amount of contaminant during the re-mounting process.

3.5 Toxicity of Suspected Biocide Residues within the NMW Herbarium

The herbarium was closed to the public and staff in 2001 following the suggestion that common symptoms amongst key staff members, such as dry eyes, itchy skin, sore throat and sinus problems, were related to close work on the herbarium material. Previous research linked medical and health complaints, such as headaches, sore eyes, sore throats, chest pains, nausea, dermatitis, dizziness, vomiting and digestive problems, to pesticide applications within natural history collections (Linnie, 1990).

In order to determine the most likely pesticide residues present within the NMW herbarium, a questionnaire, based on Linnie's research and solely relating to botanical collections and pesticide, was sent out to institutions in Britain and abroad (Purewal, 1998). The recipients were requested to indicate which pesticide, from a given list, had been used in their establishment. The results (Table 4) correlated well with Linnie's survey and showed that numerous different chemicals could have been applied to the specimens within the AC-NMW collections. The accumulation of different chemicals, both organic and inorganic, could also be present, all of which can be inhaled, ingested or absorbed through the skin. As well as a human health risk, this mixture of chemicals can be particularly damaging to other materials, such as plastics (Fenn, 1999). Herbicides, in particular, use receptors to increase absorption through the plant cell wall. Mammalian skin is much more readily compromised than plant cell walls and so some herbicides are very adept at passing through human skin. This absorption can be greatly enhanced in the presence of more than one chemical (Brand and Mueller, 2002).

Staff members and some visitors, work in close contact with the collections, handling numerous specimens every day, as well as close identification work using a hand lens. Identification and quantification of the applied pesticides is imperative for risk assessment, the development of safe working protocols and appropriate mitigation procedures to reduce the contaminant to an acceptable safe level.

Table 4 Results of questionnaire regarding applied pesticide treatments from herbaria worldwide

Pesticides	Armenia (1)	Austria (1)	Belarus (1)	Canada (4)	China (1)	Estonia (1)	France (1)	Germany (1)	Hungary (1)	India (1)	UK (3)	US (6)
As_2O_3					x			x				
$BaFSiO_6$								x				
CS_2		x						x	x		x	
CCl_4												x
Dowfume				x								x
DDT			x			x						
Ethylene oxide		x			x		x					x
HCN								x				
Mystox											x	x
Lindane												
$HgCl_2$				x	x			x		x	x	x
CH_3Br		x		x	x			x			x	x
Naphthalene	x		x	x	x	x				x	x	x
PDB				x		x			x	x	x	x
Permethrin								x	x		x	
Phosphine				x				x	x			
Pyrethrum		x						x	x		x	x
Raid				x								
Thymol										x		
Dichlorvos			x			x		x			x	x

*Number in brackets following the country name relates to the number of responses from that country.

Although the extent of contamination on the sheets and the effect on air quality was unknown, preliminary studies on the NMW herbarium revealed that the herbarium sheets contained significant levels of mercury, lead, arsenic, barium and naphthalene (Purewal, 1998). The health Implications of these chemicals are given in Appendix 1. In order to determine how much contamination was in the air and hence gain an indication of possible exposure of key staff members working closely on the collections, the NMW undertook a programme of air quality monitoring within the herbarium and biological monitoring of certain staff.

3.5.1 Measurements of Mercury Vapour Within the NMW Herbarium Environment

Anchem Analytical Laboratories were employed to monitor the air quality within the botany division by measuring the concentration of mercury within the working environment (Fig.17).
An air pump (Fig.18) calibrated by standard bubble meter was employed to draw air through the monitor at a rate of 2 litres per minute for a period of three hours. The air was drawn through a 0.8µm mixed-cellulose ester membrane (MCE) filter that bound any mercury present in the air. The filter was removed, digested in acid and analysed using cold vapour-atomic absorption spectrometry (CV-AAS). Nine sample sites were analysed, including work areas and the interior of herbarium cabinets. Although mercury was detected, all of the sites showed levels below 0.0001mg/m^3, well below the maximum levels recommended in the Health and Safety Executive guidelines (0.02mg/m^3 (SCOEL, 2003), TWA 0.02mg/m^3 (Quinn, 2005)).

Figure 17 Technician from Anchem Laboratories monitoring the inside of a cabinet

Figure 18 Passive air sampling pump used to measure mercury present within the cabinets.

Work carried out within the Cambridge University herbarium (Briggs *et al.*, 1983), showed that mercury levels in air could be significantly reduced by ventilation: a drop from 0.025mg/m^3 to 0.01mg/m^3 was achieved at Cambridge after a ventilation system was installed within the herbarium.

It is apparent that with the low levels of mercury found within the herbarium atmosphere that inhalation is not the key route of entry. It is most probable that hand to mouth contamination, ingestion from contaminated dusts and/or skin absorption through handling the collections, are the main routes of exposure for staff.

3.5.2 Measurements of Naphthalene Vapour within the NMW Herbarium Environment

The University of Wales College of Medicine, Health, Safety and Environment Unit (UWMC) were commissioned to monitor the naphthalene concentration within the NMW herbarium environment during 2002. Tubes packed with an appropriate highly adsorbent sampling substrate (Tenax TA®) were placed in cabinets and in areas of main activity and attached to the clothing of staff members within their breathing zone. Both passive and active methods of sampling were employed. The passive technique required ambient airflow across the sampling substrate. The active method employed a SKC Sidekick pump running at a flow rate of 150ml/minute to draw the air through the sampling substrate. The naphthalene was analysed by thermal desorption and capillary gas chromatography with flame ionisation detection, carried out by the Environmental Measurement Group at the Health and Safety Laboratory in Sheffield.

The results are given in Tables 5 and 6. UWMC determined that naphthalene concentrations within the cabinets (0.46–0.62mg/m^3) were significantly higher than those determined in the open working areas (<0.05mg/m^3). The National Toxicological Program (NTP) study states that 9µg/m^3 (NIEHS, 2004) causes respiratory problems in mice. This is below the detection limit (<50µg/m^3) of the analyses carried out in the NMW herbarium, although the personnel monitors indicated that staff were being exposed to significantly greater concentrations on average, over a working day (170–330µg/m^3). The odour threshold of naphthalene is reported to be between 99µg/m^3 (USEPA, 1990, von Rotberg, Gagelmann *et al.*, 2005) and 360µg/m^3 (NIOSH, 1981), which is consistent with the TWA staff exposure observed, as, even with the herbarium doors kept closed for the three months prior to analysis, naphthalene odour was still detectable within the herbarium environment.

Table 5 Naphthalene concentrations in air by passive sampling of personnel and work areas.

Date	Type of sample	Exposure time (min)	Sampling location	Naphthalene (mg/m^3)
02.01.02	Static	360	Botany Hb Cabinet 51	0.46
02.01.02	Static	360	Botany room 67	0.09
03.01.02	Static	420	Botany staff office	<0.05
03.01.02	Static	420	Botany Hb outside cabinets	<0.05
03.01.02	Static	420	Botany Hb work table	<0.05
08.02.02	Static	360	Botany Hb Cabinet 57	0.5
08.02.02	Static	360	Botany Hb Cabinet 57	0.62

Table 6 Naphthalene concentrations determined from personnel monitoring, indicating the time weighted average (TWA).

Date	Type of sample	Exposure time (min)	Staff member	Naphthalene TWA (mg/m^3)
02.01.02	Personnel	360	B	0.17
02.01.02	Personnel	360	C	0.23
02.01.02	Personnel	360	E	0.27
02.01.02	Personnel	360	F	0.33

The current HSE naphthalene standard in air is 10ppm (HSE, 2005) (53mg/m^3 v/v, 11.9mg/m^3 m/m). The levels detected within the cabinets (max=0.62mg/m^3) and within the open working environment (<0.05mg/m^3) and the time weighted average exposure to staff (max=0.33mg/m^3) were all significantly less than the HSE standard. However, as the herbarium had been closed for several months and access to the cabinets was restricted prior to analysis, the levels of naphthalene within the open working areas were much reduced. Once normal working practice is resumed, it would be expected that the naphthalene levels within the herbarium would increase. Working on the collections (opening cabinets and working through specimens that are contaminated with naphthalene) is likely to increase exposure to staff.

The half life of naphthalene is reportedly less than one day in the presence of UV light (Mackay, 1992, Pelish, Slusher *et al.*, 2003). It would, therefore, be expected that levels of naphthalene would naturally drop over time in the presence of light. However, although the herbarium has very large windows, each is fitted with UV filters, which would effectively prevent the photochemical breakdown. This natural degradation process would also not occur within a closed environment, where light is excluded (Howard, 1989). Intervention would therefore be required to reduce staff exposure to naphthalene.

3.5.3 Biological Monitoring of NMW herbarium workers

Six members of NMW staff were monitored to determine if mercury and arsenic were accumulating within the tissues. Although hair is the most simple means to determine long-term contamination by mercury and is recommended by the US Environmental Protection Agency (USEPA), recent studies have found it to be unreliable (Kataeva, Panichev *et al.*, 2008). As urine is the most common biological sample for monitoring levels of mercury exposure, reflecting the cumulative dose (Clarkson, 1986), samples of urine were taken and analysed for both mercury and arsenic. In order to monitor short-term exposure (5-day), blood samples were also taken and analysed. For accurate analyses, it was important that the tested staff avoided fish for seven days before the test, as fish can be a high source of heavy metal and could interfere with the final result. The analyses were repeated after 16 months, following the implementation of safe handling procedures. The results (Table 7) showed that two members of staff (A and D) had elevated levels of mercury and arsenic, slightly higher than the normal levels set by the Super Regional Assay Laboratories, in accordance with the Health and Safety Executive, but not sufficiently high to cause serious alarm (Purewal, 1998, Purewal, 1999, Spears, Purewal *et al.*, 2007). Both of these staff members handled a large number of specimens every day. Staff member D complained of itchy and sore skin on the hands, which could have been due to absorption of arsenic through the skin, or naphthalene, which was also present. Subject A provided test results for both time periods but showed, in effect a halving of the original elevated values, after implementation of safe standard procedures.

Table 7 Arsenic and mercury levels within blood and urine samples of six herbarium workers at the NMW. Normal levels for mercury and arsenic in urine have been defined as 8.1 ppm and <10 ppm, respectively. Levels for industrial concern are defined as 32.5 ppm and 10–50 ppm for mercury and arsenic, respectively. Normal blood mercury levels are defined as <20nmol/L.

Staff Member (Test date)	**Urine As/creatinine (ppm)**	**Urine Hg /creatinine (ppm)**	**Blood Mercury (nmol/L)**
A (1998)	12	6	Not tested
A (2000)	5	3	7
B (1998)	1	0.6	Not tested
B (2000)	2	1.4	3
C (1998)	Stated as Normal	Stated as Normal	Not tested
C (2000)	Not detected	1.3	4
D (1998)	11	Not tested	Not tested
D (2000)	Not tested	Not tested	Not tested
E (1998)	4	0.6	Not tested
E (2000)	8	0.9	5
F (1998)	6	1.4	Not tested
F (2000)	1	2.4	6

Similar studies carried out in two herbaria in South Africa (Kataeva et al., 2008) compared key herbarium staff workers with non-herbarium workers and found that the control group of 20 people had a mean mercury/creatinine concentration of 1.25µg/g, whilst the average herbarium workers' level was 1.75 µg/g. This information indicates that the average levels within herbarium workers was c. 0.55 µg/g higher than in those not exposed to a contaminated workplace. However, these levels are well within the suggested background mercury levels of 5µg/g creatinine (WHO, 1991).

This study confirmed that herbarium workers can become contaminated through handling and working with herbarium collections and that even when safe standard procedures have been implemented, contamination levels are not always reduced. Heavy metals are readily excreted through urine, however, if exposure remains high, then these metals will start to be deposited in the tissues. It is therefore important to monitor staff regularly to determine their base-line level of heavy metal concentration.

Furthermore, Kataeva *et al* measured the mercury content of dust and plaster from around a herbarium and found that both contributed significantly to the mercury levels within the herbarium environment. The dust mercury levels ranged from 46.2-437µg/g and the mercury content of the plaster was found to be 6.9-59.4 µg/g (Kataeva *et al.*, 2008). It is evident that good housekeeping is needed to reduce these levels, as this particulate material can be ingested and provide a further source of contamination to workers and visitors alike.

Following the studies carried out at the NMW, immediate changes were made to herbarium working practice within. Information was posted around the herbarium informing staff and visitors of the problem and how to reduce contamination. Such control methods included:

- Wearing of nitrile gloves (powder free) – an excellent barrier to the majority of historical biocides. After use the glove should be removed by pulling inside out without contaminating the clean hands and thrown away;
- Wearing of lab coats only in the contaminated zones;
- Ensuring areas are well ventilated before commencing work;
- Limiting time working within the suspected contaminated areas;
- Stopping close work such as working with a hand-held lens and using a microscope;
- Carrying out as much work as possible within a filter fume cabinet;
- Forbidding the consumption of food and drink in areas of suspected contamination; and
- Washing of hands before eating, drinking, smoking or applying make-up.

3.6 Mitigating Toxicity within Herbarium Collections

Reducing the presence of biocide residues in collections is a priority, in order to avoid the contamination of herbarium workers and visitors through handling and inhalation. Decontamination of organic pollutants, such as naphthalene, is believed to be more readily achievable than the removal of heavy metals and has been shown to be effective (Unger, 2003, von Rotberg *et al.*, 2005). A number of the more common methods of decontamination are:

- Re-mounting material
- Use of microchamber™ products
- Vacuuming
- Compressed air
- Ultraviolet light
- Heating
- Freeze-drying
- Laser cleaning
- Supercritical extraction

The most effective method of reducing all forms of contamination from the herbarium sheets would be to re-mount every specimen (Fig.19). This has been current practice within the majority of natural history institutions, mainly to increase the longevity of the specimen by incorporating archivally-sound materials and not as a method of decontamination. Specimens are re-mounted to standardise the collections, to improve on the quality of mount board and the method of attachment, but will also remove the greater part of the contamination. However, despite the over-rising benefits, several key institutions are now questioning the need to re-mount. Not only because it is a costly and timely process, but also because historic specimens are mounted on historic papers and their removal and subsequent loss, may be considered unethical. The mount sheet holds information on the type of paper used (e.g. process, mill and watermark), giving the quality and cost of the paper. This can be indicative of the type of collector. Provenance can also be determined from the paper. In addition, there is the archival interest that includes the method of mounting, handwriting, notes, marks and stains, giving further information about the collector and his collection. To lose the sheet could be deemed as losing a part of the history of that specimen. Unfortunately, the older the specimen, the older the mount (with associated higher significance and value) and the more contaminated the paper sheet is likely to be.

Exceptions can obviously be made and procedures such as interleaving with microchamber™ papers or board, instead of re-mounting, could be effective, but still costly. Microchamber™ comes in several different forms, as packing, paper, card and even paint. It incorporates activated charcoal that adsorbs pollutants and binds them, preventing their re-release into the atmosphere. This is very effective, but the lifetime of the product is not known and so replacement may need to be quite frequent, incorporating higher costs and increased man-hours.

Figure 19 Specimens being positioned before re-mounting.

As dust gathers on a collection, it picks up toxins from contaminated objects it comes into contact with and from hazardous vapours. These dusts are easily dispersed back into the air from handling and can be ingested or inhaled. High-efficiency particulate air (HEPA) filter vacuums have been used in contaminated stores to safely remove the dust whilst preventing it from re-circulating (Bégin *et al.*, 1999, Kaminitz, 2001) which is a major disadvantage of some vacuum cleaners. Once cleaning has been completed, the contents of the HEPA vacuum can then be disposed of safely, thus reducing the particulate contamination from within a store.

From a specimen perspective, vacuuming is a little more complex. Botanical material is dry and friable and any amount of airflow can disturb plant material as well as delicate diagnostic characteristics that are not always easily visible. Vacuuming a herbarium sheet may be feasible if the vacuum has a very low suction, a narrow hose end with a filter over it, is kept a fair distance away from the specimen and the hose is directed away from the specimen. It would, however, be preferable to restrict the use of vacuums to the cabinets and storage areas, rather than the specimens directly.

The use of compressed air has been studied in the removal of crystals of PDB, naphthalene, DDT and methoxychlor on ethnographic objects at the Danish National Museum (Glastrup J., 2001). While identifying the method as practically usable, the results indicated that most of the pesticides tested remained in the objects. Removing the surface crystals did not decontaminate the object, although surface concentration was reduced, leading to a reduction in contamination from vapour emissions, dust contamination and handling issues. This method could be applied to the herbarium sheets at a safe distance from the specimen itself. This should be carried out under fume extraction.

UV light has been used effectively to reduce the concentration of some agricultural pesticides in soil. The soil is churned to expose the buried pesticide to air and sunlight, thus denaturing the toxic property of that chemical (Cress D., 1990, Saxton & Engel, 2007). Naphthalene, for example, rapidly photo-degrades on exposure to sunlight with a half-life of 3-8 hours (Pelish *et al.*, 2003). Similarly, UV light will cause chemical damage to some materials, such as herbarium specimens. Exposing

such material to UV light should, therefore, be avoided. Thus, decontamination by this method is not recommended for herbaria.

Thermolignum™ have devised a method using temperature and relative humidity to remove certain pesticide residues, such as pentachlorophenol (PCP) and lindane ($C_6H_6Cl_6$), which are prevalent in museum collections; associated with having a neurotoxic effect and are considered carcinogenic (Tello, Jelen *et al.*, 2005a). Thermolignum™ sealed a room of 72.5m^3 and heated it to a maximum of 55^oC (core temperature) for ten days. Before treatment, the air concentration of PCP ranged from 0.0011-0.0012 ppm. After decontamination the ambient air measured 0.00018-0.00036 ppm (von Rotberg *et al.*, 2005).

A separate study has shown that treated materials such as wood can hold high concentrations of PCP and organochlorines, including lindane and DDT, deep within the wood. Microwaves have been used as a method of decontamination. After 1 hour within the microwave, the surface concentration of the wood increased greatly as the organochlorines were drawn from the body of the wood to the surface. After the treatment, the wood was cut in half and was found to be greatly reduced in PCP concentration (Unger, 2003). This could be an effective decontamination method for robust collections, but adequate venting of the area should be considered.

Using temperature on herbarium material could be an issue, as the specimen is sensitive to temperatures above 50^oC. Such temperatures could affect seed viability and DNA extraction. Microwaves are reported not to be damaging to the plant specimen due to the minimal amount of water present within the specimen (Hall, 1981). However, if there is any residual water, the stem of the plant has been seen to split in half from the pressure of the steam (Hill, 1983). It is also unlikely that the seed viability or the genetic information would remain intact.

Freeze-drying has been used in an attempt to remove organochlorine pesticide residues from hen eggs (Zabik and Dugan, 1971). The compounds tested were lindane, dieldrin, DDT and DDT-DDD. However, the vapour pressure of the various residues and the amount of contamination present, affected the success of the technique.

Laser (light amplification by stimulated emission of radiation) cleaning has been used in conservation for many years and is proving to be extremely effective at removing encrusted surface dirt. In most cases only the dirt is removed and the original surface is left intact, including the original patina on metal objects. It has been used on papers to clean away dirt and on cultural objects to remove contaminants. However, lasers use both heat and light irradiation and little Is known of the long-term effects of these treatments, nor of the secondary effects caused by localised heating and light irradiation (Abraham, 1999). The use of lasers on paper may cause a change in the mechanical properties (Kolar, Strlic *et al.*, 2000) and the occurrence of extreme temperatures and light intensities may cause irreversible alteration of the paper matrix (Rudolph, Ligterink *et al.*, 2004).

A further decontamination method, recently devised, is that of using supercritical carbon dioxide,

which is a particularly powerful solvent and has been used successfully on some organic compounds and inorganic residues, such as mercuric chloride and arsenic trioxide. Arsenic was not effectively removed, but mercury was reduced between 70 and 90%. DDT was removed by almost 100% (Tello, Jelen *et al.*, 2005b).

There are several techniques that could be applied to museum collections and in particular herbarium specimens, but the efficiency of some of the more economical techniques is questionable. For higher success rates, the cost of technology increases and so does the amount of damage that the specimen can be subjected to.

In summary, a cost effective, non destructive technique is required that is capable of removing not only surface contamination but is able to draw out locked-in contaminants from within the cellulose matrix to aid the ever-growing problem of removing these hazardous chemicals from the working environment.

4 Proposed Research

4.1 Rationale

The health of herbarium staff workers was a cause for concern within the NMW herbarium, as staff members working on the collections for more than five years, began showing similar signs of ill health. For example, loss of smell, sinusitis, rhinitis, eczema, itchy dry skin, dry eyes, nose and throat.

There has been much published material on the presence of historical pesticide residues on zoological material, which was first investigated following the observation of a white powder on the surface of mounted taxidermy specimens. The consequences of working with and handling this material became an issue with regards to health and safety, especially as material such as this was frequently on open display and sent out on loan to schools.

The same concerns should have been applied to botanical collections, but since the treatments were not always visible, health concerns were not initially raised. At the NMW, only naphthalene had been applied to the collections, a chemical that had never given staff members any cause for concern. Further studies into the health effects of naphthalene (see Appendix 1) have shown that it is an unpleasant substance that can cause many skin, eye, nose and throat complaints and is also a suspected carcinogen. Naphthalene can be detected by odour at very low levels and this is therefore a good method of identifying its presence. However, it is quite common for herbarium workers to be completely unable to smell naphthalene after working with it for some time.

A priority for the NMW herbarium was to ascertain the level of contamination of naphthalene within the collections and to devise a successful method of decontamination.

A condition survey of the NMW herbarium revealed that, although the majority of the collections were in very good condition, some of the sheets had observable stains surrounding the specimen. On discussion with curators that had worked within the herbarium for many years, the assumption was an application of mercuric chloride had probably been applied to the specimen. Knowing the toxicological concerns surrounding mercury (see Appendix 1) it was essential that the sheets were tested to ascertain whether this was fact and therefore a potential risk to health from working on the collections.

Using sensitive, analytical equipment is very time consuming, expensive and frequently difficult to carry out and it was not feasible to test the whole collection. It was also difficult to ensure that any sheets selected from the collection for testing had been treated historically with a biocide, as relevant data was rarely present. Consequently, the need for a simple method of identifying which specimen sheets had been exposed to a biocide treatment became paramount.

Simple spot tests and flame tests were carried out to see if these could provide any information regarding past treatments, but all were found to be too ambiguous. During examination under

ultraviolet light (366nm), the areas around the botanical specimen were seen to fluoresce. Cellulose, especially degraded cellulose, is known to fluoresce a blue/white colour due to oxidised degradation products, or peach from the fungal attack of foxing, or green from iron gall ink decay. The colours observed on the herbarium sheets were very different and appeared to be water stains and droplets. It is possible that degradation was being catalysed by metal ions present from the paper-making process, or applied as a biocide to the sheets. This could accelerate cellulose degradation via the Fenton reaction in the same way as iron and copper. As the fluorescent marks were apparent on the majority of sheets inspected, it was possible they were related to historic biocide treatments. If this were the case, the fluorescence could provide a simple method for identifying whether the herbarium sheets had been treated and therefore contaminated. This information would enable safe standard procedures to be implemented to protect personnel and also provide a rapid, effective method of identifying contaminated samples within the collections and provide a means to prioritise which collections require immediate re-mounting. As there are often several hundred thousand specimens within a herbarium, this would be a beneficial and timesaving resource within a large collection. It would also enable the removal of a large amount of hazardous chemical from the herbarium environment and allow for safe disposal.

4.2 Aims and Objectives

The overall aim of this research is to establish methodologies to reduce the amount of toxic residues present within herbarium collections. To this end, two main aims are defined:

- To develop a rapid and cost-effective screening method for identifying toxic residues on herbarium sheets, in order to prioritise a re-mounting schedule and
- To establish the most suitable decontamination method for the removal of naphthalene from herbarium collections.

In order to achieve these aims, the following objectives are defined:

- To establish any correlation between the emissions wavelengths of fluorescent areas on herbarium mount sheets with the presence and speciation, of heavy metals associated with historical biocide treatments (mercury, arsenic, lead and barium). The use of type material is particularly appropriate for this, as information on provenance will allow any information gained to be transferred to other institutions, where similar practices will have occurred.
- To carry out accelerated ageing tests to determine whether the observed fluorescence is due to historical biocide application, an artefact of degradation, or a combination of both.
- To provide information on how naphthalene behaves with a cellulose matrix such as paper. For example, does the vapour purely re-crystallise onto the paper surface or is it absorbed deeper into the papers fibres?
- To evaluate three different methods of mitigation of naphthalene from herbarium collections: freeze-drying, heating and airing. The main considerations are efficacy, economy, speed, ease of application and maintaining the integrity of the specimen.

PART II: INORGANIC BIOCIDE RESIDUES

5 Experimental

5.1 Sample Selection and Preparation

The samples for analysis were selected carefully from three different sources:

- The NMW type collection
- External herbarium specimens from collections worldwide▪
  The non-type NMW material.

Once this material was gathered, an element of random selection was employed to ensure that the material was varied and not from the same folders. Non-type material was included to provide information on the general material within the NMW herbarium and to provide a broader spectrum of dates, paper weight, locality and collector. The samples ranged in date from the mid 1700s to 1977. Samples other than the herbarium sheets were also chosen to act as controls such as original herbarium papers and folders including herbarium sheets[3], type folders[4], photocopy paper[5] and actual botanical specimens.

Five hundred and eight samples were analysed from one hundred and five herbarium sheets. All sheets used for analysis were cellulose-based, i.e. were cotton, linen or wood pulp paper (Table 8). Most of the material was specifically selected from aged type collections, and regardless of date and provenance, the majority of sheets showed fluorescing areas. It was assumed that non-fluorescing areas were either not treated with a biocide or the chemical sequence of events leading to fluorescence had not reached an end point.

3 Herbarium sheet Conqueror High Speed white board calcium carbonate buffered Wiggins Teape

4 Type Folder Lignin free. Printed red Wiggins Teape

5 Photocopy paper Volumax, SA

Table 8 Summary of the herbarium mount sheets selected for the analysis

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
01a	2290	Yellow	0.356	109	1872-1916	7.5Y 9/4	x	x
01b	2290	Clean	0.333	109	1872-1916	n/a	x	x
01c	2290	White/violet	0.171	109	1872-1916	5P 9/2	x	x
01d	2290	Clean	n/a	109	1872-1916	n/a	x	x
01e	2290	Clean	n/a	109	1872-1916	n/a	x	x
01f	2290	Cream (was Yellow)	n/a	109	1872-1916	5P 8/1	x	x
01g	2290	White	n/a	109	1872-1916	5P 9/1	x	x
02a	28.131.2296	Clean	0.446	205	1894	n/a	x	x
02b	28.131.2296	Peach	0.541	205	1894	7.5YR 8/10	x	x
02c	28.131.2296	White	0.534	205	1894	5P 9/1	x	x
02d	28.131.2296	Clean	n/a	205	1894	n/a	x	x
02e	28.131.2296	Peach	n/a	205	1894	7.5YR 8/10	x	x
03a	27.72.2352	Clean	0.205	90	1878	n/a	x	x
03b	27.72.2352	Peach	0.324	90	1878	7.5YR 8/10	x	X
03c	27.72.2352	Brown	n/a	90	1878	10YR 7/6	x	X
03d	27.72.2352	Clean	n/a	90	1878	n/a	x	X
03e	27.72.2352	Grey	n/a	90	1878	10BG 5/1	x	X
03f	27.72.2352	Orange (Foxing)	n/a	90	1878	10YR 9/4	x	x
03g	27.72.2352	Peach (Foxing)	n/a	90	1878	7.5YR 8/10	x	x
03h	27.72.2352	Peach (Foxing)	n/a	90	1878	7.5YR 8/10	x	x
03i	27.72.2352	White	n/a	90	1878	5P 9/1	x	x
04a	49.29.1683	Cream	0.498	127	1930	5P 8/1	x	x
04b	49.29.1683	Clean	0.496	127	1930	n/a	x	x
04c	49.29.1683	Clean	n/a	127	1930	n/a	x	x
04d	49.29.1683	Peach	n/a	127	1930	7.5YR 8/10	x	x
05a	46.221.1	Clean	0.407	131	1945	n/a	✓	Isotype
05b	46.221.1	Yellow	0.480	131	1945	7.5Y 9/4	✓	Isotype
05c	46.221.1	Brown (Foxing)	n/a	131	1945	10YR 7/31	✓	Isotype
05d	46.221.1	Clean (was White)	n/a	131	1945	n/a	✓	Isotype
05e	46.221.1	Clean (was Yellow)	n/a	131	1945	n/a	✓	Isotype

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
06a	V94.24.7131	Grey	0.565	247	1938	10BG 5/1	x	x
06b	V94.24.7131	Clean	0.486	247	1938	n/a	x	x
06c	V94.24.7131	Clean	n/a	247	1938	n/a	x	x
06d	V94.24.7131	Cream	n/a	247	1938	5P 8/1	x	x
06e	V94.24.7131	Grey	n/a	247	1938	10BG 5/1	x	x
07a	Not Accessed	Clean	0.447	192	1933	n/a	x	x
07b	Not Accessed	Peach	0.632	192	1933	7.5YR 8/10	x	x
07c	Not Accessed	Clean	n/a	192	1933	n/a	x	x
07d	Not Accessed	Cream	n/a	192	1933	5P 8/1	x	x
07e	Not Accessed	White	n/a	192	1933	5P 9/1	x	x
07f	Not Accessed	Yellow	n/a	192	1933	7.5Y 9/4	x	x
08a	Not Accessed	White	0.326	195	1923	5P 9/1	x	x
08b	Not Accessed	Clean	0.364	195	1923	n/a	x	x
08c	Not Accessed	Yellow	0.477	195	1923	7.5Y 9/4	x	x
08d	Not Accessed	Clean	n/a	195	1923	n/a	x	x
08e	Not Accessed	Cream (was Yellow)	n/a	195	1923	5P 8/1	x	x
08f	Not Accessed	Peach (was Brown)	n/a	195	1923	7.5YR 8/10	x	x
09a	Not Accessed	Grey	0.456	170	1909	10BG 5/1	✓	x
09b	Not Accessed	Clean	0.521	170	1909	n/a	✓	x
09c	Not Accessed	Clean	n/a	170	1909	n/a	✓	x
09d	Not Accessed	Brown	n/a	170	1909	10BG 5/1	✓	x
10a	V94.24.679	Clean	0.400	94	1833	n/a	✓	x
10b	V94.24.679	Brown	0.302	94	1833	10YR 7/9	✓	x
10c	V94.24.679	Grey	0.302	94	1833	10BG 5/2	✓	x
10d	V94.24.679	Clean	n/a	94	1833	n/a	✓	x
10e	V94.24.679	Clean	n/a	94	1833	n/a	✓	x
10f	V94.24.679	Grey	n/a	94	1833	10BG 5/1	✓	x
11a	Not Accessed	Clean	n/a	199	1935	n/a	x	x
11b	Not Accessed	Grey	n/a	199	1935	10BG 5/1	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
11c	Not Accessed	Orange	n/a	199	1935	10YR 9/4	x	x
11d	Not Accessed	Peach	n/a	199	1935	7.5YR 8/10	x	x
11e	Not Accessed	Yellow	n/a	199	1935	7.5Y 9/4	x	x
12a	86.81.3428	Yellow	0.551	160	1944	7.5Y 9/4	x	Holotype
12b	86.81.3428	Clean	0.332	160	1944	n/a	x	Holotype
12c	86.81.3428	Clean	n/a	160	1944	n/a	x	Holotype
12d	86.81.3428	Peach	n/a	160	1944	7.5YR 8/10	x	Holotype
13a	23.94.1936	Peach	0.355	87	1915	7.5YR 8/10	x	Paratype
13b	23.94.1936	Clean	0.392	87	1915	n/a	x	Paratype
14a	25.79.2834	Brown (Foxing)	n/a	144	1915	10YR 7/31	✓	Paratype
14b	25.79.2834	Clean	n/a	144	1915	n/a	✓	Paratype
15a	27.72.3187a	Clean	0.339	148	1892	n/a	x	x
15b	27.72.3187a	Yellow	0.596	148	1892	7.5Y 9/4	x	x
15c	27.72.3187a	Clean	n/a	148	1892	n/a	x	x
15d	27.72.3187a	Grey	n/a	148	1892	10BG 5/1	x	x
15e	27.72.3187a	Yellow	n/a	148	1892	7.5Y 9/4	x	x
17a	No longer in collection	Clean	0.512	147	1933	n/a	✓	x
17b	No longer in collection	Peach	0.399	147	1933	7.5YR 8/10	✓	x
17c	No longer in collection	Clean	n/a	147	1933	n/a	✓	x
17d	No longer in collection	Cream	n/a	147	1933	5P 8/1	✓	x
17e	No longer in collection	Peach (Foxing)	n/a	147	1933	7.5YR 8/10	✓	x
18a	No longer in collection	Clean	0.342	104	1861	n/a	✓	x
18b	No longer in collection	Grey	0.294	104	1861	10BG 5/1	✓	x
18c	No longer in collection	Clean	n/a	104	1861	n/a	✓	x
18d	No longer in collection	Grey	n/a	104	1861	10BG 5/1	✓	x
18e	No longer in collection	Peach	n/a	104	1861	7.5YR 8/10	✓	x
19a	Not Accessed	Clean	0.442	198	1930	n/a	x	x
19b	Not Accessed	White	0.209	198	1930	5P 9/1	x	x
19c	Not Accessed	Yellow	0.502	198	1930	7.5Y 9/4	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
19d	Not Accessed	Orange	0.483	198	1930	10YR 9/4	x	x
19e	Not Accessed	Orange	n/a	198	1930	10YR 9/4	x	x
19f	Not Accessed	Yellow	n/a	198	1930	7.5Y 9/4	x	x
19g	Not Accessed	Clean	n/a	198	1930	n/a	x	x
19h	Not Accessed	Clean	n/a	198	1930	n/a	x	x
19i	Not Accessed	Yellow	n/a	198	1930	7.5Y 9/4	x	x
19j	Not Accessed	Orange	n/a	198	1930	10YR 9/4	x	x
20a	28.131.2163	Clean	n/a	112	1895	n/a	✓	Lectotype
20b	28.131.2163	Grey	n/a	112	1895	10BG 5/1	✓	Lectotype
20c	28.131.2163	White	n/a	112	1895	5P 9/1	✓	Lectotype
21a	28.131.2163	Clean	n/a	175	1895	n/a	x	Lectotype
21b	28.131.2163	Grey	n/a	175	1895	10BG 5/1	x	Lectotype
21c	28.131.2163	Orange	n/a	175	1895	10YR 9/4	x	Lectotype
21d	28.131.2163	Peach	n/a	175	1895	7.5YR 8/10	x	Lectotype
22a	423.64	Clean	n/a	123	1972	n/a	x	x
22b	423.64	Cream	n/a	123	1972	5P 8/1	x	x
22c	423.64	Orange glue	n/a	123	1972	10YR 9/4	x	x
22d	423.64	Peach	n/a	123	1972	7.5YR 8/10	x	x
23a	86.81.2123	Clean	n/a	111	1922	n/a	x	Isoparatype
23b	86.81.2123	Grey	n/a	111	1922	10BG 5/1	x	Isoparatype
24a	V92.36.19	Clean	0.432	159	1954	n/a	✓	Holotype
24b	V92.36.19	Grey	0.435	159	1954	10BG 5/1	✓	Holotype
24c	V92.36.19	Clean	n/a	159	1954	n/a	✓	Holotype
24d	V92.36.19	Grey	n/a	159	1954	10BG 5/1	✓	Holotype
25a	V92.36.19	Clean	0.463	106	1954	n/a	✓	Holotype
25b	V92.36.19	Peach	0.399	106	1954	7.5YR 8/10	✓	Holotype
26a	V92.36.19	Peach	0.451	160	1954	7.5YR 8/10	✓	Holotype
26b	V92.36.19	Clean	0.601	160	1954	n/a	✓	Holotype
27a	423.9	Clean	0.238	125	1931	n/a	x	x
27b	423.9	white/violet	0.101	125	1931	5P 9/1	x	x
28a	18.125	Brown	n/a	107	1895	10YR 7/9	x	x
28b	18.125	Clean	n/a	107	1895	n/a	x	x
29a	25.7a.412	Orange	0.168	153	1915	10YR 9/4	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
29b	25.7a.412	Clean	0.221	153	1915	n/a	x	x
29c	25.7a.412	Clean (was white)	n/a	153	1915	n/a	x	x
29d	25.7a.412	Clean (was Cream	n/a	153	1915	n/a	x	x
30a	86.81.686	Grey	0.441	142	1945	10BG 5/1	x	Paratype
30b	86.81.686	Clean	0.336	142	1945	n/a	x	Paratype
30c	86.81.686	Cream	0.407	142	1945	5P 8/1	x	Paratype
30d	86.81.686	Clean	n/a	142	1945	n/a	x	Paratype
30e	86.81.686	Clean	n/a	142	1945	n/a	x	Paratype
30f	86.81.686	Cream	n/a	142	1945	5P 8/1	x	Paratype
30g	86.81.686	Grey	n/a	142	1945	10BG 5/1	x	Paratype
30h	86.81.686	Peach (Foxing)	n/a	142	1945	7.5YR 8/10	x	Paratype
33a	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33b	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33c	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33d	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33e	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33f	no longer in collection	Clean	n/a	91	1948	n/a	x	x
33g	no longer in collection	Peach	n/a	91	1948	7.5YR 8/10	x	x
34a	No longer in collection	Clean	0.420	88	1950	n/a	x	x
34b	No longer in collection	Grey	0.385	88	1950	10BG 5/1	x	x
36a	No longer in collection	Clean	0.297	84	1833	n/a	x	x
36b	No longer in collection	Grey	0.515	84	1833	10BG 5/1	x	x
36c	No longer in collection	Peach	0.958	84	1833	7.5YR 8/10	x	x
39a	Not Accessed	Clean 1	n/a	108	1955	n/a	✓	x
39b	Not Accessed	Clean 2	n/a	108	1955	n/a	✓	x
39c	Not Accessed	Clean 3	n/a	108	1955	n/a	✓	x
39d	Not Accessed	Clean 4	n/a	108	1955	n/a	✓	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
39e	Not Accessed	Clean 5	n/a	108	1955	n/a	✓	x
39f	Not Accessed	Clean 6	n/a	108	1955	n/a	✓	x
39g	Not Accessed	Missed the White spot	n/a	108	1955	n/a	✓	x
39h	Not Accessed	White 1	n/a	108	1955	5P 9/1	✓	x
39i	Not Accessed	White 2	n/a	108	1955	5P 9/1	✓	x
39j	Not Accessed	White 3	n/a	108	1955	5P 9/1	✓	x
39k	Not Accessed	White 4	n/a	108	1955	5P 9/1	✓	x
39l	Not Accessed	White 5	n/a	108	1955	5P 9/1	✓	x
39m	Not Accessed	White 6	n/a	108	1955	5P 9/1	✓	x
40a	28.131.6954	Brown	n/a	172	1909	10YR 7/10	x	Paralectotype
40b	28.131.6954	Clean	n/a	172	1909	n/a	x	Paralectotype
40c	28.131.6954	Clean (was white)	n/a	172	1909	n/a	x	Paralectotype
40d	28.131.6954	Cream	n/a	172	1909	5P 8/1	x	Paralectotype
42a	23.94.1254	Clean 1	n/a	147	1917	n/a	✓	Paratype
42b	23.94.1254	Clean 2	n/a	147	1917	n/a	✓	Paratype
42c	23.94.1254	Clean 3	n/a	147	1917	n/a	✓	Paratype
42d	23.94.1254	Clean 4	n/a	147	1917	n/a	✓	Paratype
42e	23.94.1254	Clean 5	n/a	147	1917	n/a	✓	Paratype
42f	23.94.1254	Clean 6	n/a	147	1917	n/a	✓	Paratype
42g	23.94.1254	Orange Fox 1	n/a	147	1917	10YR 9/4	✓	Paratype
42h	23.94.1254	Orange Fox 2	n/a	147	1917	10YR 9/4	✓	Paratype
42i	23.94.1254	Orange Fox 3	n/a	147	1917	10YR 9/4	✓	Paratype
42j	23.94.1254	Orange Fox 4	n/a	147	1917	10YR 9/4	✓	Paratype
42k	23.94.1254	Orange Fox 5	n/a	147	1917	10YR 9/4	✓	Paratype
42l	23.94.1254	Orange Fox 6	n/a	147	1917	10YR 9/4	✓	Paratype
45a	Not Accessed	White (was Clean)	n/a	105	1962	5P 9/1	x	Paratype
45b	Not Accessed	White/Indigo	n/a	105	1962	5P 9/1	x	Paratype
46a	86.81.3431	Brown	n/a	239	1945	10YR 7/11	✓	Isotype
46b	86.81.3431	Clean	n/a	239	1945	n/a	✓	Isotype
46c	86.81.3431	Peach	n/a	239	1945	7.5YR 8/10	✓	Isotype

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
47a	86.81.3431	Peach Fox 1	n/a	108	1945	7.5YR 8/10	✓	Isotype
47b	86.81.3431	Peach Fox 2	n/a	108	1945	7.5YR 8/10	✓	Isotype
47c	86.81.3431	Peach Fox 3	n/a	108	1945	7.5YR 8/10	✓	Isotype
47d	86.81.3431	Peach Fox 4	n/a	108	1945	7.5YR 8/10	✓	Isotype
47e	86.81.3431	Peach Fox 5	n/a	108	1945	7.5YR 8/10	✓	Isotype
47f	86.81.3431	Peach Fox 6	n/a	108	1945	7.5YR 8/10	✓	Isotype
48a	28.131.2454	Brown	n/a	134	1893	10YR 7/12	✓	Isotype
48b	28.131.2454	Clean	n/a	134	1893	n/a	✓	Isotype
48c	28.131.2454	Peach	n/a	134	1893	7.5YR 8/10	✓	Isotype
48d	28.131.2454	Yellow	n/a	134	1893	7.5Y 9/4	✓	Isotype
49a	57.380.52	brown	n/a	140	1908	10YR 7/13	✓	Isoparatype
49b	57.380.52	brown	n/a	140	1908	10YR 7/13	✓	Isoparatype
49c	57.380.52	Clean	n/a	140	1908	n/a	✓	Isoparatype
49d	57.380.52	Clean	n/a	140	1908	n/a	✓	Isoparatype
50a	166.5	Cream	0.361	167	1952	5P 8/1	✓	x
50b	166.5	Clean	0.392	167	1952	n/a	✓	x
51a	152.4	Clean	0.313	152	1898	n/a	✓	x
51b	152.4	Cream	0.409	152	1898	5P 8/1	✓	x
51c	152.4	Peach	0.409	152	1898	7.5YR 8/10	✓	x
52a	86.81.549	Peach	0.484	138	1952	7.5YR 8/10	✓	x
52b	86.81.549	Clean	0.364	138	1952	n/a	✓	x
52c	86.81.549	Clean 1	n/a	138	1952	n/a	✓	x
52d	86.81.549	Clean 2	n/a	138	1952	n/a	✓	x
52e	86.81.549	Clean 3	n/a	138	1952	n/a	✓	x
52f	86.81.549	Clean 4	n/a	138	1952	n/a	✓	x
52g	86.81.549	Clean 5	n/a	138	1952	n/a	✓	x
52h	86.81.549	Clean 6	n/a	138	1952	n/a	✓	x
52i	86.81.549	Grey	n/a	138	1952	10BG 5/1	✓	x
52j	86.81.549	Orange	n/a	138	1952	10YR 9/4	✓	x
52k	86.81.549	Peach	n/a	138	1952	7.5YR 8/10	✓	x
52l	86.81.549	Yellow	n/a	138	1952	7.5Y 9/4	✓	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
52m	86.81.549	Yellow	n/a	138	1952	7.5Y 9/4	✓	x
52n	86.81.549	Yellow 1	n/a	138	1952	7.5Y 9/4	✓	x
52o	86.81.549	Yellow 2	n/a	138	1952	7.5Y 9/4	✓	x
52p	86.81.549	Yellow 3	n/a	138	1952	7.5Y 9/4	✓	x
52q	86.81.549	Yellow 4	n/a	138	1952	7.5Y 9/4	✓	x
52r	86.81.549	Yellow 5	n/a	138	1952	7.5Y 9/4	✓	x
52s	86.81.549	Yellow 6	n/a	138	1952	7.5Y 9/4	✓	x
53a	40.444.1	Clean	0.350	99	1940	n/a	✓	Paratype
53b	40.444.1	Yellow	0.404	99	1940	7.5Y 9/4	✓	Paratype
54a	40.444.1	Clean	0.310	99	1940	n/a	✓	Paratype
54b	40.444.1	White	0.357	99	1940	5P 9/1	✓	Paratype
55a	28.130.190	Clean	0.571	176	1893	n/a	x	Paratype
55b	28.130.190	Peach	0.758	176	1893	7.5YR 8/10	x	Paratype
56a	25.149.7892	White	0.373	115	1909	5P 9/1	✓	Lectotype
56b	25.149.7892	Grey	0.286	115	1909	10BG 5/1	✓	Lectotype
56c	25.149.7892	Clean	0.469	115	1909	n/a	✓	Lectotype
57a	27.638.978	Clean	0.760	99	1906	n/a	✓	Lectotype
57b	27.638.978	Cream	0.760	99	1906	5P 8/1	✓	Lectotype
59a	No longer in collection	Clean	n/a	162	1898	n/a	✓	x
59b	No longer in collection	Cream (missed the spot)	n/a	162	1898	5P 8/1	✓	x
59c	No longer in collection	grey	n/a	162	1898	10BG 5/1	✓	x
59d	No longer in collection	Orange glue	n/a	162	1898	10YR 9/4	✓	x
59e	No longer in collection	Specimen	n/a	162	1898	n/a	✓	x
61a	No longer in collection	Cream	0.373	214	1850	5P 8/1	✓	x
61b	No longer in collection	Grey	0.373	214	1850	5P 8/1	✓	x
61c	No longer in collection	Cream	0.286	214	1850	5P 8/1	✓	x
61d	No longer in collection	Clean	0.469	214	1850	n/a	✓	x
62a	25.149.3229	Clean	0.380	110	1892	n/a	✓	Paratype
62b	25.149.3229	Peach	0.489	110	1892	7.5YR 8/10	✓	Paratype
63a	86.81.1840	Clean	0.333	88	1955	n/a	x	Paratype
64a	86.81.1840	Clean	0.421	140	1955	n/a	✓	Paratype
64b	86.81.1840	Yellow	0.598	140	1955	7.5Y 9/4	✓	Paratype
65a	86.81.1840	Peach	0.494	140	1955	7.5YR 8/10	x	Paratype
65b	86.81.1840	Clean	0.303	140	1955	n/a	x	Paratype
65c	86.81.1840	Cream	0.187	140	1955	5P 8/1	x	Paratype
66a	86.81.1840	Grey	0.727	140	1955	10BG 5/1	x	Paratype
66b	86.81.1840	Clean	0.413	140	1955	n/a	x	Paratype
66c	86.81.1840	Cream	0.346	140	1955	5P 8/1	x	Paratype
66d	86.81.1840	Clean	0.346	140	1955	n/a	x	Paratype
66e	86.81.1840	Grey	0.346	140	1955	10BG 5/1	x	Paratype

Table 8 (contiued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
67a	49.29.1685	Clean	0.362	112	1935	n/a	✓	x
67b	49.29.1685	Yellow	0.481	112	1935	7.5Y 9/4	✓	x
69a	29.435.43	Clean	0.407	137	1929	n/a	✓	Isoparatype
69b	29.435.43	Cream	0.420	137	1929	5P 8/1	✓	Isoparatype
71a	28.131.7962	Cream	0.677	155	1897	5P 8/1	x	Isotype
71b	28.131.7962	Clean	0.378	155	1897	n/a	x	Isotype
71c	28.131.7962	Peach	0.311	155	1897	7.5YR 8/10	x	Isotype
72a	28.131.7963	Clean	n/a	155	1977	n/a	x	Isotype
72b	28.131.7963	Orange	n/a	155	1977	10YR 9/4	x	Isotype
72c	28.131.7963	White	n/a	155	1977	5P 9/1	x	Isotype
73a	25.149.1068	Brown	n/a	111	1894	10YR 7/15	✓	x
73b	25.149.1068	Clean	n/a	111	1894	n/a	✓	x
75a	31.344.22	Clean	0.359	200	1931	7.5YR 8/10	✓	x
75b	31.344.22	Peach	0.246	200	1931	n/a	✓	x
76a	31.344.25	Orange	0.483	204	1931	10YR 9/4	✓	x
76b	31.344.25	Clean	0.462	204	1931	n/a	✓	x
76c	31.344.25	Yellow	0.471	204	1931	7.5Y 9/4	✓	x
76d	31.344.25	Cream	0.471	204	1931	7.5Y 9/4	✓	x
76e	31.344.25	Cream	n/a	204	1931	5P 8/1	✓	x
76f	31.344.25	Orange	n/a	204	1931	10YR 9/4	✓	x
76g	31.344.25	Peach	n/a	204	1931	7.5YR 8/10	✓	x
76h	31.344.25	Yellow	n/a	204	1931	7.5Y 9/4	✓	x
77a	25.79.1398	Orange	0.456	132	1889	10YR 9/4	✓	x
77b	25.79.1398	Clean	0.435	132	1889	n/a	✓	x
77c	25.79.1398	White	0.230	132	1889	5P 9/1	✓	x
77d	25.79.1398	Peach	0.471	132	1889	7.5YR 8/10	✓	x
78a	23.94.2534	Clean	n/a	142	1908	n/a	✓	Paralecto-type
78b	23.94.2534	Grey	n/a	142	1908	10BG 5/1	✓	Paralecto-type
79a	86.81.3422	Clean	n/a	139	1935	n/a	x	Holotype
79b	86.81.3422	Yellow	n/a	139	1935	7.5Y 9/4	x	Holotype
80a	86.81.549	Clean	0.55	138	1890	n/a	✓	x
81a	V.97.37.52	Clean	0.545	84	1895	NA	x	Syntype

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
Aa1a	n/a	Cream	0.583	266	n/a	5P 8/1	x	n/a
Aa1b	n/a	Cream	0.583	266	n/a	5P 8/1	x	n/a
Aa2	n/a	Clean	0.572	282	n/a	n/a	x	n/a
Aa3	n/a	Peach	0.552	272	n/a	7.5YR 8/10	x	n/a
Aa4	n/a	Clean	0.590	291	n/a	n/a	x	n/a
Aa5a	n/a	Yellow	0.585	289	n/a	7.5Y 9/4	x	n/a
Aa5b	n/a	Yellow	0.585	289	n/a	7.5Y 9/4	x	n/a
Aa5c	n/a	Yellow	0.585	289	n/a	7.5Y 9/4	x	n/a
Aa6a	n/a	Clean	0.575	284	n/a	n/a	x	n/a
Aa6b	n/a	Clean	0.575	284	n/a	n/a	x	n/a
Aa6c	n/a	Clean	0.575	284	n/a	n/a	x	n/a
Aa7a	n/a	Peach	0.578	285	n/a	7.5YR 8/10	x	n/a
Aa7b	n/a	Peach	0.578	285	n/a	7.5YR 8/10	x	n/a
Aa7c	n/a	Peach	0.578	285	n/a	7.5YR 8/10	x	n/a
Aa8a	n/a	Clean	0.585	289	n/a	n/a	x	n/a
Aa8b	n/a	Clean	0.585	289	n/a	n/a	x	n/a
Aa8c	n/a	Clean	0.585	289	n/a	n/a	x	n/a
Aa	Not Accessed	Clean	0.445	124	1932	n/a	✓	x
Ab	Not Accessed	Yellow	0.530	124	1932	7.5Y 9/4	✓	x
Ac	Not Accessed	Clean	n/a	124	1932	n/a	✓	x
Ad	Not Accessed	Cream	n/a	124	1932	5P 8/1	✓	x
Ae	Not Accessed	Clean	n/a	124	1932	n/a	✓	x
Af	Not Accessed	Yellow	n/a	124	1932	7.5Y 9/4	✓	x
Ag	Not Accessed	Peach	n/a	124	1932	7.5YR 8/10	✓	x
Air	Not accessed	n/a	n/a	n/a	n/a	n/a	x	x
Ba	26.16.42	Clean	0.360	125	1925	n/a	✓	Holotype
Bb	26.16.42	Clean	0.453	125	1925	n/a	✓	Holotype
Bu a01	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a02	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a03	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a04	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a05	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a06	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a07	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a08	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a09	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a10	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a11	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a12	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a13	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a14	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a15	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a16	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a17	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a18	26.254	n/a	n/a	103	1760s	n/a	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
Bu a19	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a20	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a21	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a22	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu a23	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b01	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b02	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b03	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b04	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b05	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b06	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b07	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b08	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b09	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b10	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b11	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b12	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b13	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b14	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b15	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu b16	26.254	n/a	n/a	103	1760s	n/a	x	x
Bu c01	26.254	Clean	0.158	103	1760s	n/a	x	x
Bu c02	26.254	Cream	0.424	103	1760s	5P 8/1	x	x
Bu c03	26.254	Grey	0.284	103	1760s	10BG 5/1	x	x
Bu c04	26.254	Clean	n/a	103	1760s	n/a	x	x
Bu c05	26.254	Grey	n/a	103	1760s	10BG 5/1	x	x
Bu c06	26.254	White	n/a	103	1760s	5P 9/1	x	x
Bu c07	26.254	White	n/a	103	1760s	5P 9/1	x	x
Ca	28.131.7153	Clean	0.475	135	1904	n/a	x	x
Cb	28.131.7153	Grey	0.478	135	1904	10BG 5/1	x	x
Cc	28.131.7153	Grey	0.473	135	1904	10BG 5/1	x	x
Cd	28.131.7153	Clean	0.475	135	1904	n/a	x	x
Ce	28.131.7153	Brown	n/a	135	1904	10YR 7/16	x	x
Cf	28.131.7153	Clean	n/a	135	1904	n/a	x	x
Cg	28.131.7153	Cream	n/a	135	1904	5P 8/1	x	x
Ch	28.131.7153	Grey	n/a	135	1904	10BG 5/1	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
Ci	28.131.7154	Grey	n/a	135	1904	10BG 5/1+G433	x	x
Da	No longer in collection	Clean	0.391	114	1848	n/a	x	x
Db	No longer in collection	Cream	0.576	114	1848	5P 8/1	x	x
Dc	No longer in collection	Clean	n/a	114	1848	n/a	x	x
Dd	No longer in collection	Yellow was Cream	n/a	114	1848	7.5Y 9/4	x	x
Ea	86.81.3421	Peach	0.411	112	1964	7.5YR 8/10	✓	Holotype
Eb	86.81.3421	Cream	0.493	112	1964	5P 8/1	✓	Holotype
Ec	86.81.3421	Clean	0.452	112	1964	n/a	✓	Holotype
Ed	86.81.3421	Clean	n/a	112	1964	n/a	✓	Holotype
Ee	86.81.3421	Clean	n/a	112	1964	n/a	✓	Holotype
Ef	86.81.3421	Grey	n/a	112	1964	10BG 5/1	✓	Holotype
F01a	USA7329A	Clean	0.487	148	1936	n/a	✓	x
F01b	USA7329A	Cream	0.595	148	1936	5P 8/1	✓	x
F01c	USA7329A	White	0.595	148	1936	5P 9/1	✓	x
F02a	USA5533	Clean	0.547	154	1937	n/a	✓	x
F03a	USA2964	Clean	0.510	147	1936	n/a	x	x
F03b	USA2964	Orange	0.581	147	1936	10YR 9/4	x	x
F04a	USA12028	Clean	1.044	154	1885	5P 8/1	x	x
F04b	USA12029	Cream	0.638	154	1885	n/a	x	x
F05a	CAN1319	Clean	0.424	75	1872	n/a	x	x
F05b	CAN1319	Peach	0.618	75	1872	7.5YR 8/10	x	x
F06a	CAN 56352	Clean	0.339	129	1882	n/a	x	x
F06b	CAN 56352	Cream	0.762	129	1882	5P 8/1	x	x
F06c	CAN 56352	Black	n/a	129	1882	10YR 2.5/1	x	x
F06d	CAN 56352	Cream	n/a	129	1882	5P 8/1	x	x
F06e	CAN 56352	Grey	n/a	129	1882	10BG 5/1	x	x
F06f	CAN 56352	Peach	n/a	129	1882	7.5YR 8/10	x	x
F06g	CAN 56352	Yellow	n/a	129	1882	7.5Y 9/4	x	x
F07a	CAN88664	Cream	0.557	163	1800	5P 8/1	x	x
F07b	CAN88664	Yellow	0.557	163	1800	7.5Y 9/4	x	x
F07c	CAN88664	Clean	0.449	163	1800	n/a	x	x
F07d	CAN88664	Brown	n/a	163	1800	10YR 7/17	x	x
F07e	CAN88664	Brown 1	n/a	163	1800	10YR 7/33	x	x
F07f	CAN88664	Brown 2	n/a	163	1800	10YR 7/34	x	x
F07g	CAN88664	Brown 3	n/a	163	1800	10YR 7/35	x	x
F07h	CAN88664	Brown 4	n/a	163	1800	10YR 7/36	x	x
F07i	CAN88664	Brown 5	n/a	163	1800	10YR 7/37	x	x
F07j	CAN88664	Brown 6	n/a	163	1800	10YR 7/38	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
F07k	CAN88664	Clean 1	n/a	163	1800	n/a	x	x
F07l	CAN88664	Clean 2	n/a	163	1800	n/a	x	x
F07m	CAN88664	Clean 3	n/a	163	1800	n/a	x	x
F07n	CAN88664	Clean 4	n/a	163	1800	n/a	x	x
F07o	CAN88664	Clean 5	n/a	163	1800	n/a	x	x
F07p	CAN88664	Clean 6	n/a	163	1800	n/a	x	x
F07q	CAN88664	Cream 1	n/a	163	1800	5P 8/1	x	x
F07r	CAN88664	Cream 2	n/a	163	1800	5P 8/1	x	x
F07s	CAN88664	Cream 3	n/a	163	1800	5P 8/1	x	x
F07t	CAN88664	Cream 4	n/a	163	1800	5P 8/1	x	x
F07u	CAN88664	Cream 5	n/a	163	1800	5P 8/1	x	x
F07v	CAN88664	Cream 6	n/a	163	1800	5P 8/1	x	x
F07w	CAN88664	Grey	n/a	163	1800	10BG 5/1	x	x
F08a	CAN86560	White	1.123	149	1891	5P 9/1	x	x
F08b	CAN86560	Clean	1.060	149	1891	n/a	x	x
F09a	CAN119529	Brown	0.882	127	1905	n/a	x	x
F09b	CAN119529	Clean	0.810	127	1905	n/a	x	x
F09c	CAN119529	Brown	n/a	127	1905	10YR 7/20	x	x
F09d	CAN119529	Brown	n/a	127	1905	10YR 7/21	x	x
F09e	CAN119529	Clean	n/a	127	1905	n/a	x	x
F10a	CAN36657	White	0.380	74	1875	5P 9/1	x	x
F10b	CAN36657	Clean	0.700	74	1875	n/a	x	x
F10c	CAN36657	Clean	n/a	74	1875	n/a	x	x
F10d	CAN36657	White	n/a	74	1875	5P 9/1	x	x
F11a	CAN100743	Brown	0.510	64	1878	10YR 7/22	x	x
F11b	CAN100743	Clean	0.255	64	1878	n/a	x	x
F11c	CAN100743	Brown	0.140	64	1878	10YR 7/25	x	x
F11d	CAN100743	Cream	0.140	64	1878	5P 8/1	x	x
F11e	CAN100743	Brown	n/a	64	1878	10YR 7/26	x	x
F11f	CAN100743	Clean	n/a	64	1878	n/a	x	x
F11g	CAN100743	Cream	n/a	64	1878	5P 8/1	x	x
F11h	CAN100743	Yellow	n/a	64	1878	7.5Y 9/4	x	x
F12a	CAN100743	Cream	0.720	64	1878	5P 8/1	x	x
F12b	CAN100743	Yellow	0.720	64	1878	7.5Y 9/4	x	x
F12c	CAN78830	Clean	0.510	63	1870	n/a	x	x
F13a	CAN75954	Clean	0.520	95	1877	n/a	x	x
F13b	CAN75954	Cream	0.190	95	1877	5P 8/1	x	x
F13c	CAN75954	Grey	0.270	95	1877	10BG 5/1	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
F13d	CAN75954	Clean 1	n/a	95	1877	n/a	x	x
F13e	CAN75954	Clean 2	n/a	95	1877	n/a	x	x
F13f	CAN75954	Clean 3	n/a	95	1877	n/a	x	x
F13g	CAN75954	Clean 4	n/a	95	1877	n/a	x	x
F13h	CAN75954	Clean 5	n/a	95	1877	n/a	x	x
F13i	CAN75954	Clean 6	n/a	95	1877	n/a	x	x
F13j	CAN75954	Grey	n/a	95	1877	10BG 5/1	x	x
F13k	CAN75954	Grey 1	n/a	95	1877	10BG 5/1	x	x
F13l	CAN75954	Grey 2	n/a	95	1877	10BG 5/1	x	x
F13m	CAN75954	Grey 3	n/a	95	1877	10BG 5/1	x	x
F13n	CAN75954	Grey 4	n/a	95	1877	10BG 5/1	x	x
F13o	CAN75954	Grey 5	n/a	95	1877	10BG 5/1	x	x
F13p	CAN75954	Grey 6	n/a	95	1877	10BG 5/1	x	x
F13q	CAN75954	White	n/a	95	1877	5P 9/1	x	x
F14a	CAN26194	Cream	0.774	141	1884	5P 8/1	x	x
F14b	CAN26194	Brown	0.774	141	1884	10YR 7/22	x	x
F14c	CAN26194	Clean	0.650	141	1884	n/a	x	x
F14d	CAN26194	Clean	n/a	141	1884	n/a	x	x
F14e	CAN26194	Cream	n/a	141	1884	5P 8/1	x	x
F14f	CAN26194	Orange	n/a	141	1884	10YR 9/4	x	x
F14g	CAN26194	Peach (missed the spot)	n/a	141	1884	7.5YR 8/10	x	x
F14h	CAN26194	Clean	n/a	141	1884	10YR 7/22	x	x
F14i	CAN26194	Brown	n/a	141	1884	10YR 7/22	x	x
F15a	CAN161001	Clean	0.525	148	1885	n/a	x	x
F15b	CAN161001	Cream	0.600	148	1885	5P 8/1	x	x
F15c	CAN161001	White	0.600	148	1885	5P 9/1	x	x
F16a	CAN180597	White	0.560	76	1819	5P 9/1	x	x
F16b	CAN180597	Clean	n/a	76	1819	n/a	x	x
F16c	CAN180597	White	n/a	76	1819	5P 9/1	x	x
F17a	HUN597046	Clean	0.420	107	1897	n/a	x	x
F17b	HUN597046	Yellow	0.620	107	1897	7.5Y 9/4	x	x
F17c	HUN597046	Clean	n/a	107	1897	n/a	x	x
F17d	HUN597046	Yellow	n/a	107	1897	7.5Y 9/4	x	x
F17e	HUN597046	Yellow	n/a	107	1897	7.5Y 9/4	x	x
F18a	HUN186418	Clean	0.686	111	1840	n/a	x	x
F18b	HUN186418	Clean	n/a	111	1840	n/a	x	x
F18c	HUN186418	Yellow	n/a	111	1840	7.5Y 9/4	x	x
F19a	HUN38137	Peach	0.248	91	1830	7.5YR 8/10	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
F19b	HUN38137	Clean	0.483	91	1830	n/a	x	x
F20a	BEL640067	Clean	0.505	147	1896	n/a	x	x
F20b	BEL640067	Cream	0.567	147	1896	5P 8/1	x	x
F20c	BEL640067	Yellow	0.567	147	1896	7.5Y 9/4	x	x
F20d	BEL640067	Cream	0.631	147	1896	5P 8/1	x	x
F20e	BEL640067	Peach	0.631	147	1896	7.5YR 8/10	x	x
F20f	BEL640067	Clean	n/a	147	1896	n/a	x	x
F20g	BEL640067	Clean	n/a	147	1896	n/a	x	x
F20h	BEL640067	Cream	n/a	147	1896	5P 8/1	x	x
F20i	BEL640067	Cream	n/a	147	1896	5P 8/1	x	x
F21a	BEL4908	Clean	0.507	153	1950	n/a	x	x
F21b	BEL4908	Cream	0.665	153	1950	5P 8/1	x	x
F21c	BEL4908	Clean	n/a	153	1950	n/a	x	x
F21d	BEL4908	Specimen	n/a	153	1950	n/a	x	x
F21e	BEL4908	Yellow	n/a	153	1950	7.5Y 9/4	x	x
F22a	BEL1893	White	0.493	173	1949	5P 9/1	x	x
F22b	BEL1893	Yellow	0.493	173	1949	7.5Y 9/4	x	x
F22c	BEL1893	Clean	0.621	173	1949	n/a	x	x
F22d	BEL1893	Clean	n/a	173	1949	n/a	x	x
F22e	BEL1893	Grey	n/a	173	1949	10BG 5/1	x	x
F22f	BEL1893	Missed White spot	n/a	173	1949	n/a	x	x
F22g	BEL1893	Specimen	n/a	173	1949	n/a	x	x
F23a	HUN2966	Yellow	0.631	127	1922	7.5Y 9/4	x	x
F23b	HUN2966	Clean	0.485	127	1922	n/a	x	x
F24a	USA6430	Clean	0.602	159	1937	n/a	x	x
F24b	USA6430	Brown	0.545	159	1937	10YR 7/30	x	x
F24c	USA6430	Clean	n/a	159	1937	n/a	x	x
F24d	USA6430	White	n/a	159	1937	5P 9/1	x	x
F25a	USA45	Clean	0.541	127	1899	n/a	x	x
F25b	USA45	White	0.567	127	1899	5P 9/1	x	x
F25c	USA45	Yellow	0.567	127	1899	7.5Y 9/4	x	x
F26a	CAN53050	Black	n/a	164	1883	10YR 2.5/1	x	x
F26b	CAN53050	Cream	n/a	164	1883	5P 8/1	x	x
F26c	CAN53050	Cream Glue	n/a	164	1883	5P 8/1	x	x
F26d	CAN53050	Grey	n/a	164	1883	10BG 5/1	x	x
F26e	CAN53050	Grey (was Clean)	n/a	164	1883	10BG 5/1	x	x

Table 8 (continued)

Sample	Accession no	Fluoresence	Wt (g)	Density g/m²	Date	Munsell colour ref	Lignin	Type status
F26f	CAN53050	Orange	n/a	164	1883	10YR 9/4	x	x
Hb a	Not accessed	n/a	0.526	165	1970	n/a	x	x
Hb b	Not accessed	n/a	n/a	165	1970	n/a	x	x
Pc a	Not accessed	n/a	0.412	80	2000	n/a	✓	x
Pc b	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc c	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc d	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc e	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc f	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc g	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Pc h	Not accessed	n/a	n/a	80	2000	n/a	✓	x
Plastic	Not accessed	n/a	n/a	n/a	n/a	n/a	x	x
Type a	Not accessed	n/a	0.444	217	1970	n/a	✓	x
Type b	Not accessed	n/a	n/a	217	1970	n/a	✓	x

Total number of sheets = 109
Total number of sample = 526

NOTE:

- Sample numbers beginning with "F" denote specimens donated for this research from foreign institutions
- Sample 17 Labelled as "poisoned" but no other information available
- Sample 49 Labelled as "poisoned in 1909"
- Sample F17 Labelled as "treated with mercuric chloride"
- Sample F18 Labelled as "treated with carbon disulphide and phosphine"
- Sample F20 Labelled as "treated with mercuric chloride"

All specimens were removed from their mount sheets and stored with corresponding data for re-mounting. All the original data was removed from the original sheet either by removing the label from the sheet or by cutting around it and then this information was transferred onto the original sheet by hand, in pencil. The original material was then re-mounted to standardise the collections, bring all the data together and to improve the conservation standards of the sheets. The archival mount board was 100% acid free rag paper with a gelatine size. The re-mounted specimens were attached with gelatine backed linen tape, which is a completely reversible method of attachment, whilst original data was re-attached with archival glues (EvaCon®)[6].

The phluroglucinol test was applied to the sample sheets to determine the presence of lignin (Table 8). Phluroglucinol (1g) was dissolved in a mixture of methanol (50ml), concentrated hydrochloric acid (50ml) and distilled water (50ml). A drop of this solution was placed onto a test area of the sheet. A bright or deep magenta colour indicated the presence of mechanical or semi-mechanical wood pulp, unbleached chemical pulp or other lignified fibres, such as jute (Barrow 1969).

The sample sheets were examined under ultraviolet light (366nm), using a hand held UVGL-58 mineral light® lamp (Ultraviolet production inc. San Gabriel USA), to observe the fluorescent areas. These areas were identified and marked by circling with pencil. The colour of the fluorescence was determined by eye, and then referenced against a standard colour chart: the Munsell Colour Book (Munsell, 1969). The colour of the observed fluorescence (Table 8) was annotated to the side of the circled area. Areas that were not seen to fluoresce were classified as "clean" (this does not suggest that contamination was not present, only that no fluorescence was observed). Clean samples were also pencil marked and annotated. These would provide background information on the paper itself and were undoubtedly contaminated with naphthalene. The clean samples were, therefore, used as controls and would provide background information on the paper substrate for analytical purposes.
The various circles were cut out and weighed, measured and placed in labelled tubes. A minimum of two samples were taken from each sample sheet (one coloured and one clean sample). A total of 526 samples were prepared for this analysis.

6 EvaCon Ethylvinylacetate Conservation by Design, Bedfordshire.

5.2 Trace Element Analysis

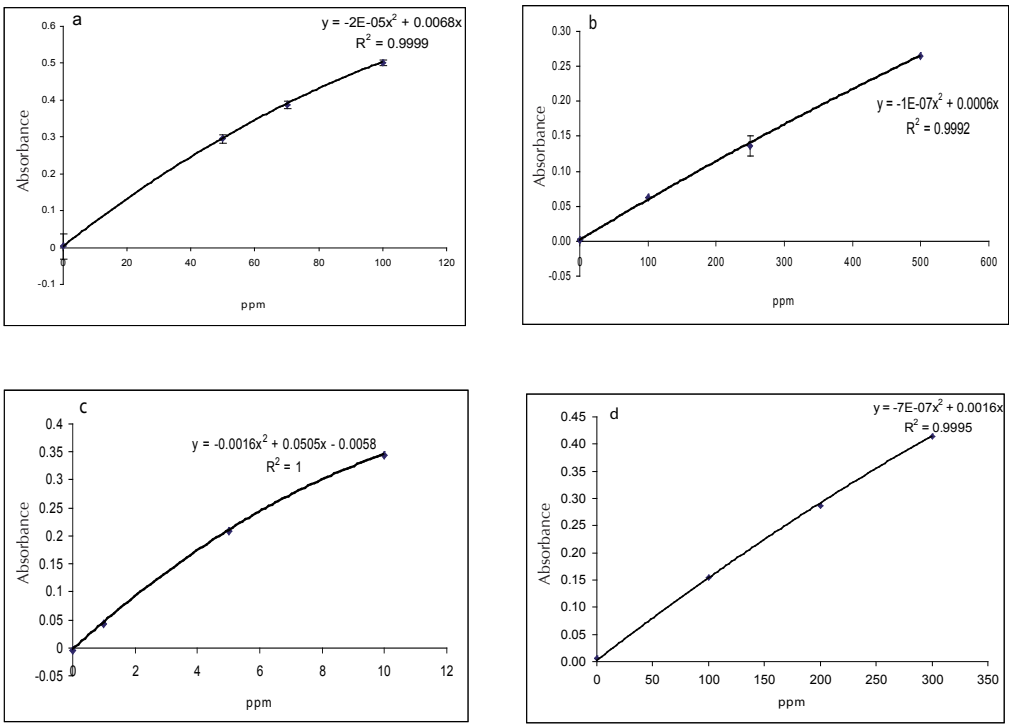
Two methods of trace element analysis were employed: atomic absorption spectrometry (AAS), which is a destructive technique requiring the sample to be in solution, and particle (proton) induced x-ray emission (PIXE), which is micro-destructive, requiring no sample preparation, and is proven to be particularly successful at analysing paper (Eldred *et al.*, 1984, Kusko and Schwab, 1987, Bulow *et al.*, 2001).

5.2.1 Atomic Absorption Spectrometry

79 sheets were analysed by AAS, representing the full colour range of fluorescent areas. Each sheet was cut up into individual samples, totalling 187 samples, which were then weighed, measured and placed in a labelled tube with concentrated nitric acid[7] (trace element grade, 2ml). The samples were heated to speed up digestion (a heated test tube holder and Infra red lamp) ensuring the mouths of the tubes were sealed with Parafilm to prevent evaporation and loss of metallic vapour. Once the samples were fully digested, they were made up to 10ml with distilled water. The resultant solutions were then centrifuged (1 minute). The samples were analysed for mercury, arsenic, lead and barium using a Unicam 919 Atomic Absorption Spectrometer with an acetylene and air flame. The instrument was set for continuous aspiration and hollow cathode lamps were used for all four elements. The sensitivity was measured at 2.3mg/litre (2.3 ppm).

7 Nitric acid Romil Pure chemistry, Romil-spa super purity acid, MW 63.01

Standards were run for each of the four elements using AAS standard solutions[8] (1000ppm), and calibration graphs were attained (Figs. 20).



Figures 20 Standard calibration curves for the analysis of (a) arsenic, (b) barium, (c) lead and (d) mercury by atomic absorption spectrometry

5.2.2 Particle Induced X-Ray Emission (PIXE)

64 sheets of herbarium paper were cut into samples representing the full colour range of fluorescent areas. This totalled 312 samples, which were then subjected to PIXE analysis at the AGLAE laboratory within the Centre de Recherche et de Restauration des Musee de France (C2RMF), in the Louvre, Paris[9]. Analyses were carried out using an NEC 2 MV tandem pelletron accelerator (Figure 21).

The samples were placed on an X,Y,Z-motorised stage and held in a plastic envelope with a hole punched over the circled fluorescent area (Figure 22). A positioning system, with CCD camera and laser, was used to place the zone of interest in the beam, at a 3mm distance from the exit window. The proton beam (diameter c.30µm) was aligned parallel to the surface normal and two Si (Li) detectors were placed at 45° – one with an ultra thin polymer window optimised for the lower energy X-rays (1–10 keV), determining the lighter elements up to Z = 22; the other with a 50µm Al filter for higher energy X-rays, determining the heavier elements. A third detector measured gamma emissions from fluorine to verify the presence of barium fluorosilicate.

8 Barium contains 997µg/ml of Ba in 1.1 wt % HCl, arsenic contains 998µg/ml As in 2% KOH, mercury contains 997µg/ml of Hg in 1 wt % HNO3, lead contains 997µg/ml of Pb in 1 wt % HNO3, Sigma-Aldrich Ltd. Gillingham, Dorset.

9 Funding was provided by EuArtech (Access Research and Technology for the Conservation of the European Cultural Heritage)

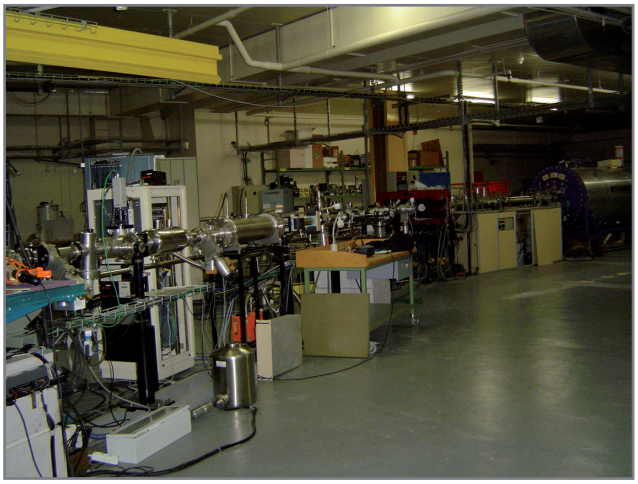


Figure 21 The tandem pelletron accelerator at the Centre de Recherche et de Restauration des Musee de France, the Louvre.

To ensure that barium could be detected, the intensity of the proton beam was increased from 3 MeV to 4 MeV for the first run of samples. This was to achieve a stronger signal from the Ba K line. Unfortunately, problems were encountered with running the instrument at 4 MeV, and all subsequent analyses had to be carried out at 3 MeV.

The samples were analysed with a proton dose varying from $2x10^5$ to $2x10^6$ counts per second. The proton dose was monitored by measuring the Si X-ray signal emitted from the 0.1 µm silicon nitride exit window with a peltier-cooled silicon drift detector. Silicon drift detectors were originally designed as position-sensitive detectors for particle tracking. Currently they are used for high-count-rate X-ray spectroscopy, operating close to room temperature (Strüder *et al.*, 1998).

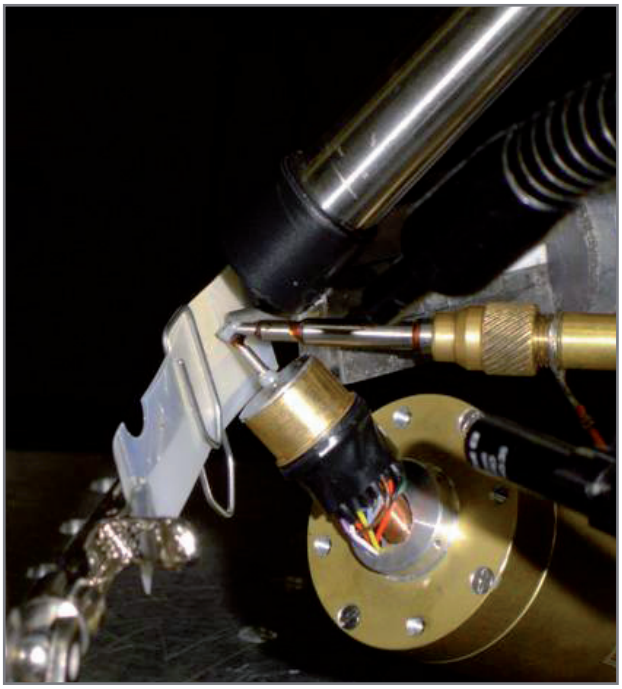


Figure 22 Remote control area showing precise positioning of sample in front of beam Musee de France, the Louvre.

Quantification was achieved using the GUPIX software (Maxwell *et al.*, 1995), calibrated against Micromatter™ (Co) X-Ray fluorescence thin film-standards (Table 9).

Samples were treated as thin samples, and hence no correction was made for X-ray absorption by the matrix (Duval *et al.*, 2004). The accuracy of this quantification is expected to be around 15–20%, mainly due to errors in dose monitoring (±10%) and the accuracy of the standard material (±5%).
Multi-elemental spot analyses (100 x100µm using the controlled stage) were carried out on all fluorescent areas. Elemental area and line mapping (500µm^2, 1000µm apart, over a distance of 4cm) was used to determine variation in the Hg, Pb and As content across the sample, incorporating fluorescent and non-fluorescent areas, and areas with visible staining. In order to eliminate elements inherent in the paper, clean samples were also analysed as controls.

Table 9 X-ray energies for all standards used

Element	Kα X-ray energies (keV)	Kβ X-ray energies (keV)	Lα X ray energies (keV)
11 Na	1.041	–	–
12 Mg	1.254	–	–
13 Al	1.487	1.560	–
14 Si	1.740	1.839	–
15 P	2.010	2.135	–
16 S	2.308	2.464	–
17 Cl	2.622	2.816	–
19 K	3.314	3.590	–
20 Ca	3.692	4.013	–
22 Ti	4.511	4.934	–
24 Cr	5.415	5.949	–
25 Mn	5.899	6.493	–
26 Fe	6.405	7.061	–
27 Co	6.930	7.651	–
28 Ni	7.478	8.263	–
29 Cu	8.048	8.906	0.930
30 Zn	8.639	9.573	1.012
31 Ga	9.252	10.264	1.100
33 As	10.544	11.726	1.282
34 Se	11.222	12.496	1.379
35 Br	11.924	13.292	1.481
37 Rb	13.395	14.961	1.694
38 Sr	14.165	15.835	1.806
40 Zr	15.775	17.667	2.042
48 Cd	23.174	26.095	3.134
50 Sn	25.271	28.486	3.444
56 Ba	32.194	36.378	4.466
80 Hg	–	–	9.989
82 Pb	–	–	10.551
83 Bi	–	–	10.839

5.3 Mercury Speciation: X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to determine the valence state of mercury in the fluorescent samples, with a view to identifying any correlation between speciation and emission wavelength of the observed fluorescence.

XPS is a micro-destructive surface technique using high-energy electrons, emitted from a hot filament, to bombard a metal foil, such as aluminium. Soft x-rays are then emitted from the foil (Al K_a radiation: $hv = 1486.6$ eV) that interacts with the first 10nm of the surface substrate being analysed. The technique measures the kinetic energy (KE) of the emitted electrons, allowing the difference in energy between the ionised (A^+) and neutral (A) atoms to be determined. This is generally called the binding energy (BE):

$$KE = hv - BE$$

$$BE = (E(A^+) - E(A))$$

h = value for Planck's Constant $6.6260755 \times 10^{-34}$ Js

v = the frequency of the photon being studied

Every element has a characteristic binding energy, giving rise to a characteristic peak during analysis. The intensity of the peak is related to the concentration of the element within the sample (Nix, 1997).

Eleven samples were submitted for XPS analysis[10] (Table 10). The samples were mounted on to VG sample holders using double-sided sticky-tape.

Table 10 Summary of samples analysed by XPS, giving the colour of the observed fluorescence and date of specimen.

Sample	Colour of Fluorescence	Date
19h	Clean	1930
19i	Yellow	1930
19j	Orange	1930
Ae	Clean	1932
Af	Yellow	1932
Ag	Peach	1932
F14h	Clean	1884
F14i	Brown	1884
AA1*	Cream	n/a
AA3*	Peach	n/a
AA4*	Clean	n/a

*see Section 5.4: Accelerated Ageing Tests

10 The analysis was carried out by Dr D. Morgan, School of Chemistry, Cardiff University.

Standard mercury compounds were run (Hg_2I_2, $HgCl_2$ and HgO) to gain reference binding energies for both Hg(I) and Hg(II), under the same spectrometer conditions.

After charge correction (approximately 2.6 eV for each set of spectra), the binding energies obtained for the Hg(4f) 5/2 peaks (±0.2 eV) were as follows:

Hg (I) 100.2 eV
Hg (II) 101.0 eV

The 1eV difference between the two oxidation states was sufficient to be resolved by the three XPS instruments utilised.

Instrument 1 (Kratos Scientific, Manchester)

An Axis ULTRA 'DLD' X-ray photoelectron spectrometer, fitted with the '*Delay Line Detector*', replacing the dual channeltron and phosphor screen detection system of previous ULTRA instruments, with a single detector used for both spectroscopic analysis and fully quantifiable, pulse counting, parallel XPS imaging. Where necessary, the automatic charge neutraliser system was used, and the spectrum calibrated to 285.0 eV binding energy for the C(1s) peak, post acquisition.

Instrument 2 (Department of Chemistry, University of Cardiff)

A VG ESCA-LAB 200i photoelectron spectrometer, using the aluminium k alpha line, with associated photon energy of 1486.6 eV. All spectra were calibrated to C(1s), with a binding energy of 284.5 eV.

Instrument 3 (Department of Chemistry, University of Cardiff)

A Kratos Axis Ultra DLD photoelectron spectrometer (Fig. 23), using a pass energy of 40 eV for narrow scans and 160 eV for survey spectra. Charge neutralisation was effected, to help alleviate charging effects, using a Kratos patented snorkel system. All spectra were calibrated using the C (1s) photoelectron line, with a binding energy of 284.7 eV.

Figure 23 XPS Instrument 3, based at Cardiff University

5.4 Accelerated Ageing Tests

Accelerated ageing tests were carried out in an attempt to reproduce the development of fluorescent areas on paper, under controlled experimental conditions.

Accelerated ageing has been used as a predictive method of determining what will happen to cellulose-based products over time, under ambient conditions. There are, however, numerous parameters that can affect the results and cause misinterpretation. For example, different types of cellulose will undergo different kinds of degradation at different rates (Baranski *et al*., 2000).

There are four main methods commonly used for accelerated ageing tests on paper, including moisture, heat, light and pollutants (Arnold, 2003). The technique increases the exposure of paper to a specific degradative factor, accelerating the relevant degradation processes. For example, UV and visible light are catalysts for paper degradation through the formation of conjugated double bonds which cause disruption of the cellulose chain (Zheng and Yao, 2006); a 10°C rise in temperature has been observed to double the number of chemical reactions that occur in paper. This effect has been known since the 19th century (Wurster, 1888). It is also acknowledged that moisture is essential in the ageing process, as it is needed to initiate acid hydrolysis (Zou *et al*., 1996).

For the purpose of this research, it was decided that heat would be the method employed for the accelerated ageing tests (Arnold, 2006) with samples conditioned at an ambient relative humidity. This would most closely reproduce ageing at ambient conditions.

The standard test method ASTM/ISR 1994 (ASTM/ISR, 1994) for Accelerated Temperature Ageing of Paper by Dry Oven Exposure Apparatus was used.

The paper chosen for the tests was pure cotton cellulose filter paper (Whatman No.2). The biocide applications used followed recipes closely resembling authentic historic applications.

Twenty four samples, (all samples were prepared in triplicate) of Whatman No 2 pure cotton cellulose were weighed and conditioned for 24 hours at 22°C and 48% RH. Biocide treatments were applied to some of the samples using naphthalene and the 1917 Kew Mixture recipe (see Table 1).

The Kew Mixture was prepared by dissolving phenol[11] (0.125g) and mercury (II) chloride[12] (0.125g) in industrial methylated spirits[13] (5.683 ml). The solution was mixed and then dripped over the paper and allowed to spread until the entire paper was wet. The chemicals were then allowed to dry for a few minutes within a fume cupboard.

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11 Phenol BDH Laboratories, Poole, Dorset. Max. limit of impurities 0.15%

12 Mercuric chloride Hopkin and Williams, Chadwell Heath, Essex.

13 IMS 99% IDA 99, Univar Ltd, Bedwas, Newport, NP1 8DR.

The naphthalene treatment was carried out by placing naphthalene crystals (0.125 g) in the bottom of a head space vial (20 ml), the sample placed on top, and the vial sealed.
The samples were prepared, as shown in Table 11, and sealed in head space vials (20ml).

Table 11 Summary of samples subjected to accelerated ageing tests. All tests were carried out in triplicate

Sample	Heated	Treated with mercuric chloride	Treated with naphthalene	No treatment
AA1	✓	✓		
AA2	✓		✓	
AA3	✓	✓	✓	
AA4	✓			✓
AA5		✓		
AA6			✓	
AA7		✓	✓	
AA8				✓

The accelerated ageing tests (80ºC for 72 hours) were carried out on samples AA1–AA4 by the University of Udine, Italy. The original seals were found to be unsuitable for the high temperature, and the tubes had to be resealed within a resin block. Samples AA5–AA8 were left sealed, under ambient conditions, at AC-NMW for 12 months.

Once the accelerated ageing tests were complete, all samples were viewed under infrared (IR) light and any observations recorded. The samples (AA1 -AA4) remained sealed, under ambient conditions, for 12 months until their return to AC-NMW. They were then viewed under UV-A light and any observations recorded. Samples AA1, AA3 and AA4 were subjected to XPS analysis.

5.5 Laser Ablation Decontamination Tests

The use of laser cleaning for the removal of biocide residues from herbarium samples, was assessed by applying the method to an important collection, the 3rd Earl of Bute's Herbarium. The specimen sheet used, dated from the mid 1700s and was treated at some point with mercuric chloride. It was believed that the residue chemically changed over time, producing mercuric sulphide (metacinnabar), which appears as a dark grey deposit over the front and back of the sheet. The samples were tested initially on blank paper and then by using the very edge of the paper to establish the optimum intensity for minimal damage. The intensity chosen was 155mJ/cm^2 with a repetition rate of 10 Hz.

Once the correct intensity was determined (11233 shots) the Bute sample was treated with the laser, ensuring that the laser was moved over the paper surface quickly and smoothly so that the surface was heated but not visibly damaged. The laser-cleaned area measured 7 x 5cm. The laser used was a Phoenix Classic Conservation Laser, based on a Q-switched, Nd: YAG laser from Lynton Lasers Limited using near infrared radiation at a wavelength of 1064nm.

6 Results

6.1 Applying a Hand-held UV Lamp

The hand-held UV lamp was found to be extremely effective at highlighting fluorescent areas not observable by eye. Under UV light, areas beneath the specimen were seen to fluoresce, disclosing areas that appeared to have droplets or splashes from an aqueous application (Fig. 24 right). The fluorescence regularly observed was of the same five different colours: white, cream, yellow, peach and orange. Black, grey and brown colours were also observed, but did not fluoresce. The black and brown were frequently observed by eye in visible light, but sometimes the UV light intensified the grey colour.

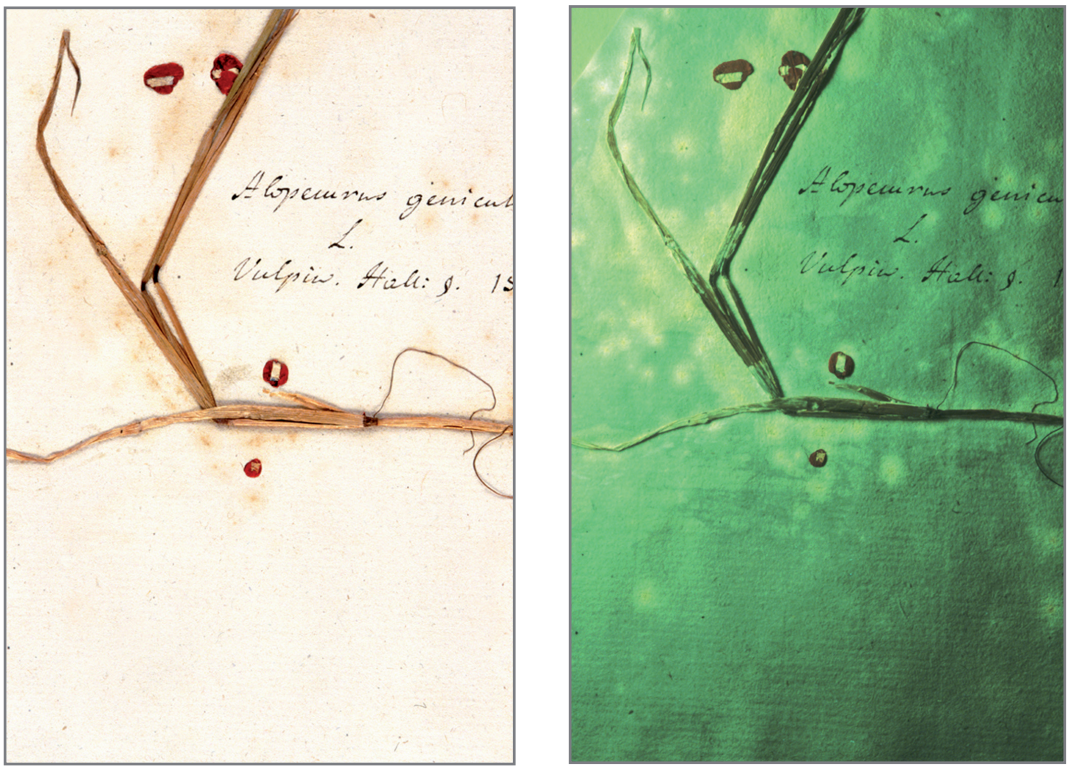

Figure 24 A herbarium sheet with specimen under visible (left) and ultraviolet light (right)

6.2 Trace Element Analysis

The results for the trace element analysis of the herbarium samples are given in Tables 12 and 13, following analysis by AAS and PIXE, respectively.

Sample sheets F17d,e,f and F20f,g,h,i had hand written data stating that mercuric chloride had been applied.

Table 12 Concentrations of four heavy metals in the herbarium samples as determined by AAS

Sample number	Fluorescence	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g
01a	Yellow	0.356	<dl	37.30	<dl	<dl*
01b	Clean	0.333	94.0	184	0.62	910
01c	White/violet	0.171	183.00	403.80	<dl	390.70
02a	Clean	0.446	nd*	144.50	4.21	112.30
02b	Peach	0.541	<dl	130.50	<dl	<dl
02c	White	0.534	354.10	132.20	9.06	93.80
03a	Clean	0.205	183.20	64.80	1.01	n/t
03b	Peach	0.324	nd	203.70	<dl	51.50
04a	Cream	0.498	11.20	126.40	<dl	33.50
04b	Clean	0.496	<dl	151	<dl	<dl
05a	Clean	0.407	<dl	128.40	1.63	<dl
05b	Yellow	0.48	<dl	127.90	0.92	<dl
06a	Grey	0.565	77.60	106.00	2.95	76.00
06b	Clean	0.486	<dl	133	17.2	68.7
07a	Clean	0.447	98.10	137.40	<dl	<dl
07b	Peach	0.632	109.10	104.40	<dl	79.30
08a	White	0.326	115.20	188.40	1.91	102.40
08b	Clean	0.364	86.0	177	0.57	91.7
08c	Yellow	0.477	170.90	138.30	<dl	175.20
09a	Grey	0.456	68.6	29.1	2.74	62.7
09b	Clean	0.521	60.1	124	0.40	32.0
10a	Clean	0.404	61.90	152.00	84.35	165.40
10b	Brown	0.302	82.90	218.50	74.22	165.80
10c	Grey	0.302	82.90	218.50	74.22	165.80
12a	Yellow	0.551	56.80	103.10	0.75	60.73
12b	Clean	0.332	75.4	180	<dl	60.6
13a	Peach	0.355	105.80	160.10	<dl	853.70
13b	Clean	0.392	2569	157	1.06	1166
15a	Clean	0.339	16.4	78.7	<dl	232
15b	Yellow	0.596	<dl	146.90	7.50	28.00
17a	Clean	0.512	24.40	122.90	0.81	97.80
17b	Peach	0.399	78.40	169.20	<dl	<dl
18a	Clean	0.342	<dl	34.5	1.29	209
18b	Grey	0.294	<dl	229.70	<dl	<dl
19a	Clean	0.442	<dl	138.90	<dl	<dl
19b	White	0.209	119.70	315.70	<dl	159.70
19c	Yellow	0.502	<dl	140.60	0.44	<dl
19d	Orange	0.483	457.30	152.50	1.29	69.10
24a	Clean	0.432	<dl	17.1	<dl	133
24b	Grey	0.435	57.50	23.70	0.51	197.50

Table 12 (continued)

Sample number	Fluorescence	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g
25a	Clean	0.463	<dl	19.1	192	172
25b	Peach	0.399	2046.80	22.20	73.06	179.40
26a	Peach	0.451	<dl	9.80	0.49	<dl
26b	Clean	0.601	<dl	12.3	1.84	167
27a	Clean	0.238	93.5	62.1	<dl	346
27b	White/violet	0.101	<dl	653.30	<dl	<dl
29a	Orange	0.168	223.60	79.10	2.47	85.10
29b	Clean	0.221	25.10	80.30	5.01	323.90
30a	Grey	0.441	<dl	13.40	<dl	162.30
30b	Clean	0.336	<dl	35.10	<dl	255.70
30c	Cream	0.407	15.40	150.90	<dl	<dl
34a	Clean	0.42	<dl	28.10	0.53	187.50
34b	Grey	0.385	<dl	38.40	1.15	n/t
36a	Clean	0.297	105.40	196.50	318.83	<dl
36b	Grey	0.515	60.80	116.30	305.36	64.80
36c	Peach	0.958	39.20	62.50	138.21	17.40
50a	Cream	0.361	121.40	161.70	nd	46.20
50b	Clean	0.392	95.8	172	1.06	42.5
51a	Clean	0.313	60.00	215.70	0.66	<dl
51b	Cream	0.409	76.50	165.10	6.65	40.80
51c	Peach	0.409	76.50	165.10	6.65	40.80
52a	Peach	0.484	116.50	114.30	0.86	69.00
52b	Clean	0.364	n/t	170.95	0.61	<dl
53a	Clean	0.35	<dl	15.12	n/t	207.46
53b	Yellow	0.404	108.50	152.00	0.51	
54a	Clean	0.31	17.86	7.2	n/t	184.04
54b	White	0.357	15.56	10.46	n/t	200.49
55a	Clean	0.571	76.80	28.50	7.44	189.20
55b	Peach	0.758	49.60	37.20	10.13	n/t
56a	White	0.373	100.70	15.80	1.67	38.30
56b	Grey	0.286	153.30	46.50	2.18	100.00
56c	Clean	0.469	66.70	31.50	1.89	<dl
57a	Clean	0.76	n/t	4.93	3.19	113.36
57b	Cream	0.486	77.30	21.30	1.37	n/t
61a	Cream	0.373	151.20	47.60	10.69	115.00
61b	Grey	0.373	151.20	47.60	10.69	115.00
61c	Cream	0.286	197.20	230.70	30.52	116.70
61d	Clean	0.469	106.80	153.80	13.06	71.20
62a	Clean	0.38	98.8	35.0	nd	37.6
62b	Peach	0.489	76.80	39.30	<dl	58.50
63a	Clean	0.333	<dl	31.0	<dl	172

Table 12 (continued)

Sample number	Fluorescence	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g
64a	Clean	0.421	74.30	28.00	8.48	n/t
64b	Yellow	0.598	73.30	24.70	2.44	47.80
65a	Peach	0.494	<dl	115.10	<dl	33.80
65b	Clean	0.303	124	198	<dl	189
65c	Cream	0.187	29.7	320	<dl	89.2
66a	Grey	0.727	n/t	2.00	<dl	n/t
66b	Clean	0.413	nd	14.3	3.22	n/t
66c	Cream	0.346	90.40	29.80	<dl	82.60
67a	Clean	0.362	15.4	32.6	<dl	198
67b	Yellow	0.481	91.10	127.70	1.73	89.20
69a	Clean	0.407	107.70	21.70	4.43	
69b	Cream	0.42	89.40	146.20	<dl	102.20
71a	Cream	0.677	<dl	<dl	0.33	468.50
71b	Clean	0.378	<dl	11.7	0.58	801
71c	Peach	0.311	161.10	47.50	<dl	926.30
75a	Clean	0.359	52.30	60.00	6.19	159.40
75b	Peach	0.246	68.5	37.0	1.80	233
76a	Orange	0.483	977.80	18.30	<dl	148.20
76b	Clean	0.462	206	48.1	<dl	186
76c	Yellow	0.471	536.70	140.10	<dl	121.50
76d	Cream	0.471	536.70	140.10	<dl	122.50
77a	Orange	0.456	<dl	131.30	3.89	<dl
77b	Clean	0.435	<dl	148.20	16.85	<dl
77c	White	0.23	81.60	280.30	2.89	<dl
77d	Peach	0.471	536.70	140.10	<dl	121.50
80a	Clean	0.55	45.9	40.7	12.0	nd
81a	Clean	0.545	n/t	49.89	n/t	n/t
Aa	Clean	0.445	<dl	134.60	<dl	<dl
Ab	Yellow	0.53	<dl	118.70	<dl	
Ba	Clean	0.363	<dl	178	<dl	<dl
Bb	Clean	0.453	<dl	142	0.49	<dl
Buc1	Clean	0.158	277.40	379.00	<dl	<dl
Buc2	Cream	0.424	88.60	141.20	37.99	916.00
Buc3	Grey	0.284	154.30	243.10	8.09	403.70
Ca	Clean	0.475	92.3	149	1.40	60.2
Cb	Grey	0.478	11.70	115.70	3.08	70.60
Cc	Grey	0.473	nd	139.50	2.25	
Cd	Clean	0.475	92.30	148.60	1.40	60.20
Pca	na	0.412	60.70	28.70	1.07	17556.60
Da	Clean	0.391	<dl	157.00	11.40	<dl
Db	Cream	0.576	65.20	106.60	1.08	29.00

Table 12 (continued)

Sample number	Fluorescence	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g
Ea	Peach	0.411	76.10	134.60	0.51	81.20
Eb	Cream	0.493	25.40	124.60	<dl	
Ec	Clean	0.452	<dl	139	<dl	<dl
F01a	Clean	0.487	<dl	9.10	3.86	<dl
F01b	Cream	0.595	<dl	24.80	7.78	<dl
F01c	White	0.595	<dl	24.80	7.78	<dl
F02a	Clean	0.547	<dl	13.50	5.74	<dl
F03a	Clean	0.51	<dl	10.36	3.47	<dl
F03b	Orange	0.581	<dl	12.70	1.07	<dl
F04a	Clean	1.044	87.30	11.60	3.93	<dl
F04b	Cream	0.638	181.10	11.30	2.00	<dl
F05a	Clean	0.424	<dl	45.40	9.91	<dl
F05b	Peach	0.618	<dl	60.20	14.48	<dl
F06a	Clean	0.339	<dl	8.70	18.07	678.70
F06b	Cream	0.762	256.50	13.60	35.78	112.80
F07a	Cream	0.557	146.40	29.20	6.40	387.10
F07b	Yellow	0.557	146.40	29.20	6.40	387.10
F07c	Clean	0.449	99.20	49.50	11.73	674.00
F08a	White	1.123	61.40	14.50	6.03	140.60
F08b	Clean	1.06	<dl	21.00	8.65	464.80
F09a	Brown	0.882	<dl	13.40	3.08	<dl
F09b	Clean	0.81	68.80	25.60	9.43	<dl
F10a	White	0.38	181.50	7.80	1.64	<dl
F10b	Clean	0.7	71.60	40.30	21.38	<dl
F11a	Brown	0.51	73.7	8.70	53.9	<dl
F11b	Clean	0.255	<dl	28.90	15.64	<dl
F11c	Brown	0.14	402.80	42.10	30.00	<dl
F11d	Cream	0.14	402.80	42.10	30.00	<dl
F12a	Cream	0.72	174.60	6.10	25.57	<dl
F12b	Yellow	0.72	174.60	6.10	25.57	<dl
F12c	Clean	0.51	<dl	14.50	29.79	<dl
F13a	Clean	0.52	107.20	14.20	12.19	414.60
F13b	Cream	0.19	131.70	54.30	15.42	1211.00
F13c	Grey	0.27	20.60	238.70	14.89	1059.50
F14a	Cream	0.774	48.50	<dl	2.70	36.90
F14b	Brown	0.774	48.50	<dl	2.70	36.90
F14c	Clean	0.654	<dl	15.80	2.23	<dl
F15a	Clean	0.525	<dl	<dl	8.41	687.40
F15b	Cream	0.6	<dl	24.60	8.07	601.40
F15c	White	0.6	<dl	24.60	8.07	601.40
F16a	White	0.563	<dl	<dl	58.01	76.20

Table 12 (continued)

Sample number	Fluorescence	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g
F17a	Clean	0.42	<dl	21.10	3.47	<dl
F17b	Yellow	0.62	70.70	16.70	6.12	<dl
F18a	Clean	0.686	<dl	<dl	67.43	41.70
F19a	Peach	0.248	<dl	<dl	37.84	<dl
F19b	Clean	0.483	<dl	6.10	2.20	<dl
F20a	Clean	0.505	<dl	14.60	1.65	<dl
F20b	Cream	0.567	<dl	15.60	0.37	<dl
F20c	Yellow	0.567	<dl	15.60	0.37	<dl
F20d	Cream	0.631	<dl	4.70	0.66	<dl
F20e	Peach	0.631	<dl	4.70	0.66	<dl
F21a	Clean	0.507	98.90	8.70	0.82	<dl
F21b	Cream	0.665	56.50	15.50	nd	<dl
F22a	White	0.493	<dl	6.00	nd	<dl
F22b	Yellow	0.493	<dl	6.00	nd	<dl
F22c	Clean	0.621	<dl	11.90	2.35	<dl
F23a	Yellow	0.631	59.50	11.70	20.50	23375.60
F23b	Clean	0.485	91.90	12.20	11.30	1615.10
F24a	Clean	0.602	62.40	14.70	5.55	nd
F24b	Brown	0.545	57.40	10.80	4.08	<dl
F25a	Clean	0.541	69.40	8.20	<dl	nd
F25b	White	0.567	99.50	15.60	7.49	25.20
F25c	Yellow	0.567	99.50	15.60	7.49	25.20
Hb a	na	0.526	35.70	105.20	<dl	163.40
Type a	na	0.444	<dl	141.70	<dl	<dl

• n/t = not tested; dl = detection limit; n/a = nat applicable

Table 13 Concentrations of the main elements detected in the herbarium samples as determined by PIXE spot analysis (100µm²)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
01d	Clean	109	4.23	3.03	8.09	<dl	355.01	1469.69	n/t
01e	Clean	109	<dl	3.59	10.76	<dl	314.93	1474.74	n/t
01f	Cream (was Yellow)	109	16.55	3.49	8.27	<dl	400.61	1203.11	n/t
01g	White	109	53.87	3.31	<dl	<dl	570.39	1019.99	n/t
02d	Clean	205	7.27	18.78	19.66	195.13	172.54	87.37	n/t
02e	Peach	205	20.83	18.15	19.32	204.2	315.48	87.13	n/t
03c	Brown	90	49.66	<dl	30.11	<dl	361.16	246.07	n/t
03d	Clean	90	16.55	<dl	28.11	<dl	683.54	137.97	n/t
03e	Grey	90	12	<dl	10.78	<dl	548.9	179.75	n/t
03f	Orange (Foxing)	90	12.44	<dl	16.22	<dl	1389.41	94.76	n/t
03g	Peach (Foxing)	90	16.89	<dl	21.88	<dl	1067.36	95.87	n/t
03h	Peach (Foxing)	90	5	<dl	11.33	<dl	495.02	114.76	n/t
03i	White	90	31.99	<dl	11.11	<dl	274.17	102.09	n/t
04c	Clean	127	5.9	7.94	<dl	<dl	481.02	328.73	n/t
04d	Peach	127	4.56	8.34	<dl	<dl	679.41	315.52	n/t
05c	Brown (Foxing)	131	<dl	<dl	4.88	<dl	201.12	112.41	n/t
05d	Clean (was White)	131	<dl	4.12	<dl	<dl	255.45	132.83	n/t
05e	Clean (was Yellow)	131	<dl	<dl	6.1	<dl	193.95	119.12	n/t
06c	Clean	247	19.48	<dl	18.96	<dl	308.74	173.49	n/t
06d	Cream	247	251.9	<dl	19.12	<dl	234.64	405.3	n/t
06e	Grey	247	50.81	<dl	19.6	<dl	441.07	345.56	n/t
07c	Clean	192	15.32	<dl	5.79	269.95	206.93	306.69	n/t
07d	Cream	192	110.71	<dl	8.55	157.93	193.43	326.92	n/t
07e	White	192	8.03	<dl	7.51	226.58	559.96	314.51	n/t
07f	Yellow	192	9.9	<dl	6.36	249.36	229.45	331.61	n/t
08d	Clean	195	3.43	23.1	25.77	2684.77	250.24	492.53	n/t
08e	Cream (was Yellow)	195	3773.31	18.29	<dl	1551.46	407.29	528.39	n/t
08f	Peach (was Brown)	195	1163.53	22.39	28.69	1758.26	234.51	409.19	n/t
09c	Clean	170	<dl	<dl	<dl	<dl	362.76	62.35	2152.29
09d	Grey	170	<dl	<dl	<dl	<dl	358.24	63.47	1744.65
10d	Clean	94	2.89	<dl	76.99	<dl	168.9	645.99	n/t
10e	Clean	94	3.01	<dl	72.1	<dl	900.01	852.97	n/t
10f	Grey	94	8.87	<dl	123.98	<dl	625.03	1286.39	n/t
11a	Clean	199	135.07	<dl	4.38	<dl	213.12	183.58	n/t
11b	Grey	199	6.99	<dl	5.64	<dl	251.21	220.87	n/t
11c	Orange	199	1722.46	<dl	13.68	<dl	611.35	503.72	n/t

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
11d	Peach	199	345.22	<dl	7.91	<dl	535.25	360.39	n/t
11e	Yellow	199	207.48	<dl	5.13	<dl	461.51	198.47	n/t
12c	Clean	160	<dl	<dl	3.93	<dl	179.8	13.23	n/t
12d	Peach	160	<dl	<dl	3.52	<dl	93.15	12.88	n/t
14a	Brown (Foxing)	144	3.26	5.39	9.53	<dl	570.42	393.18	n/t
14b	Clean	144	<dl	6.02	8.4	<dl	459.85	473.21	n/t
15c	Clean	148	40.92	70.6	<dl	<dl	216.94	322.5	n/t
15d	Grey	148	22.71	67.38	<dl	<dl	123.16	457.67	n/t
15e	Yellow	148	22.68	73.17	<dl	<dl	217.22	347.4	n/t
17c	Clean	147	<dl	<dl	10.1	111.5	1.36	<dl	n/t
17d	Cream	147	8.81	<dl	9.15	76.8	694.36	199.08	n/t
17e	Peach (Foxing)	147	2.99	<dl	10.19	<dl	613.78	239.86	n/t
18c	Clean	104	<dl	20.18	<dl	<dl	209.08	46.14	n/t
18d	Grey	104	5.64	19.57	<dl	<dl	645.78	59.12	n/t
18e	Peach	104	41.63	28.55	<dl	<dl	738.41	109.4	n/t
19e	Orange	198	1386.74	30.96	<dl	2469.98	324.55	668.52	n/t
19f	Yellow	198	132.28	30.67	<dl	2973.63	447.03	643.62	n/t
19g	Clean	198	7.13	15.83	<dl	1335.56	162.82	348.59	n/t
20a	Clean	112	6.51	<dl	6.33	<dl	761.95	384.63	n/t
20b	Grey	112	<dl	<dl	3.03	<dl	727.62	225.06	n/t
20c	White	112	4.2	<dl	3.24	<dl	308.04	202.11	n/t
21a	Clean	175	17.79	20.77	<dl	<dl	272.58	96.62	n/t
21b	Grey	112	6.24	20.54	16.88	<dl	292.54	94.39	n/t
21c	Orange	112	3508.95	31.66	37.81	<dl	489.77	311.59	n/t
21d	Peach	112	201.19	35.14	<dl	<dl	351.28	135.55	n/t
22a	Clean	123	3.38	3.89	<dl	<dl	221.72	301.24	n/t
22b	Cream	123	4.86	4.12	<dl	<dl	266.91	278.98	n/t
22c	Orange glue	123	9.34	5.68	<dl	<dl	454.58	444.19	n/t
22d	Peach	123	25.98	5.44	9.26	<dl	512.14	336.21	n/t
23a	Clean	111	<dl	<dl	<dl	<dl	557.21	199.82	2321.08
23b	Grey	111	<dl	<dl	<dl	<dl	1563.87	185.86	2872.97
24c	Clean	159	<dl	<dl	<dl	<dl	352.45	138.74	855.03
24d	Grey	159	<dl	<dl	<dl	<dl	1224.84	148.3	1223.46
28a	Brown	107	97.83	5.85	24.11	<dl	183.41	249.74	n/t
28b	Clean	107	5.52	5.52	30.93	<dl	216.37	381.51	n/t
29c	Clean was White	153	<dl	35.62	<dl	<dl	1099.37	97.1	n/t
29d	Clean (was cream)	153	<dl	26.85	<dl	<dl	245.4	63.77	n/t
30d	Clean	142	<dl	<dl	<dl	<dl	624.5	112.89	n/t
30e	Clean	142	<dl	<dl	<dl	<dl	785.68	110	n/t
30f	Cream	142	<dl	<dl	<dl	<dl	261.12	30.31	n/t

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
30g	Grey	142	<dl	<dl	10.25	<dl	2423.98	241.7	n/t
30h	Peach (Foxing)	142	<dl	<dl	<dl	<dl	495.67	92.57	n/t
33a	Clean	91	<dl	<dl	<dl	<dl	801.22	165.75	n/t
33b	Clean	91	<dl	<dl	<dl	<dl	1035.65	58.32	n/t
33c	Clean	91	<dl	<dl	4.94	<dl	158.73	15.57	n/t
33d	Clean	91	<dl	<dl	<dl	<dl	863.85	56.14	n/t
33e	Clean	91	<dl	<dl	<dl	<dl	1091.79	72.87	n/t
33f	Clean	91	<dl	<dl	<dl	<dl	457.64	52.98	n/t
33g	Peach	91	5.76	<dl	<dl	<dl	529.52	59.59	n/t
39a	Clean 1	108	<dl	20.19	<dl	<dl	204.91	254.26	1381.39
39b	Clean 2	108	<dl	<dl	<dl	<dl	197.04	252.78	1232.04
39c	Clean 3	108	<dl	<dl	<dl	<dl	184.44	253.8	1165.83
39d	Clean 4	108	<dl	15	<dl	<dl	185.37	273.24	1236.3
39e	Clean 5	108	<dl	<dl	<dl	<dl	183.98	258.06	1234.72
39f	Clean 6	108	<dl	13.89	<dl	<dl	194.63	229.72	1052.96
39g	Missed the White spot	108	<dl	<dl	<dl	<dl	192.31	243.8	1359.63
39h	White 1	108	<dl	15.74	<dl	<dl	264.81	259.54	1902.04
39i	White 2	108	<dl	<dl	<dl	<dl	289.54	259.07	1930.65
39j	White 3	108	<dl	14.35	<dl	<dl	287.04	276.3	1782.78
39k	White 4	108	<dl	15.37	<dl	<dl	275.09	313.7	1618.24
39l	White 5	108	<dl	16.2	<dl	<dl	228.15	235.19	1364.07
39m	White 6	108	<dl	15	<dl	<dl	214.81	239.35	1340.65
40a	Brown	172	12.36	41.5	<dl	<dl	212.8	131.35	n/t
40b	Clean	172	6.51	48.68	<dl	<dl	213.68	134.88	n/t
40c	Clean (was white)	172	<dl	42.6	<dl	<dl	972.95	166.22	n/t
40d	Cream	172	<dl	12.69	<dl	<dl	27.37	<dl	n/t
42a	Clean 1	147	<dl	7.62	<dl	<dl	554.9	473.13	192.93
42b	Clean 2	147	<dl	<dl	<dl	<dl	582.04	475.31	205.31
42c	Clean 3	147	<dl	7.48	<dl	<dl	407.62	360.54	124.22
42d	Clean 4	147	<dl	<dl	<dl	<dl	533.74	485.78	195.92
42e	Clean 5	147	<dl	<dl	<dl	<dl	247.01	253.2	86.53
42f	Clean 6	147	<dl	<dl	<dl	<dl	526.19	455.24	191.43
42g	Orange Fox 1	147	<dl	<dl	<dl	<dl	663.2	627.35	336.67
42h	Orange Fox 2	147	<dl	<dl	<dl	<dl	592.04	565.71	277.35
42i	Orange Fox 3	147	<dl	<dl	<dl	<dl	569.12	555.1	277.21
42j	Orange Fox 4	147	<dl	<dl	<dl	<dl	615.44	633.33	301.02
42k	Orange Fox 5	147	<dl	<dl	<dl	<dl	571.5	539.93	266.26
42l	Orange Fox 6	147	<dl	<dl	<dl	<dl	80.82	79.73	37.21
45a	White (was Clean)	105	3.97	<dl	72.07	<dl	478.36	219.09	n/t

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
45b	White/Indigo	105	<dl	<dl	69.87	<dl	600.11	179.14	n/t
46a	Brown	239	16.4	<dl	<dl	<dl	329.67	1070.92	26.32
46b	Clean	239	<dl	<dl	<dl	<dl	302.72	648.79	38.54
46c	Peach	239	25.19	<dl	<dl	<dl	274.1	826.32	22.13
47a	Peach Fox 1	108	<dl	<dl	<dl	<dl	434.4	414.31	71.83
47b	Peach Fox 2	108	<dl	<dl	<dl	<dl	404.95	431.56	70
47c	Peach Fox 3	108	<dl	<dl	<dl	<dl	424.68	392.2	68.07
47d	Peach Fox 4	108	<dl	<dl	<dl	<dl	375.69	414.5	72.48
47e	Peach Fox 5	108	<dl	<dl	<dl	<dl	357.34	366.79	64.31
47f	Peach Fox 6	108	<dl	<dl	<dl	<dl	438.99	461.47	62.57
48a	Brown	134	14.79	120.16	72.19	<dl	160.64	556.05	n/t
48b	Clean	134	30.69	124.04	74.96	57.12	217.85	570.09	n/t
48c	Peach	134	1118.19	114.33	79.3	<dl	123.48	773.44	n/t
48d	Yellow	134	104.86	163.24	91.94	94.7	160.71	564.12	n/t
49a	brown	140	<dl	<dl	<dl	<dl	3846.29	<dl	n/t
49b	brown	140	<dl	<dl	5.82	<dl	111.01	49.26	n/t
49c	clean	140	<dl	<dl	7.21	<dl	105.18	50.28	n/t
49d	Clean	140	<dl	2.77	<dl	<dl	114.06	62.02	n/t
52c	Clean 1	138	<dl	<dl	<dl	221.38	600.29	251.52	793.55
52d	Clean 2	138	22.17	8.12	<dl	215.94	603.62	237.46	719.71
52e	Clean 3	138	<dl	<dl	<dl	188.48	635.22	301.3	770.87
52f	Clean 4	138	<dl	<dl	<dl	220.29	620.22	253.84	800.51
52g	Clean 5	138	19.93	<dl	<dl	188.48	648.7	253.48	781.74
52h	Clean 6	138	<dl	<dl	<dl	196.67	622.17	297.1	810.72
52i	Grey	138	14.6	7.02	53.89	725.28	844.05	403.91	n/t
52j	Orange	138	521.94	<dl	72.19	533.4	522.12	337.18	n/t
52k	Peach	138	43.53	<dl	66.36	544.77	1337.8	300.11	n/t
52l	Yellow	138	490.79	<dl	64.88	580.54	477.48	351.32	n/t
52m	Yellow	138	576.52	<dl	<dl	191.59	448.91	301.38	755.36
52n	Yellow 1	138	531.88	<dl	<dl	190.72	441.81	315.51	770.8
52o	Yellow 2	138	2138.99	<dl	<dl	193.33	522.68	532.17	690.43
52p	Yellow 3	138	545	<dl	<dl	227.97	432.9	324.49	833.99
52q	Yellow 4	138	113.7	<dl	<dl	177.83	437.39	304.35	858.77
52r	Yellow 5	138	2223.04	<dl	<dl	186.52	504.06	609.42	807.46
52s	Yellow 6	138	1903.12	<dl	<dl	221.88	511.01	601.74	804.06
59a	Clean	162	5.75	24.17	53.62	<dl	233.99	271.98	n/t
59b	Cream (missed the spot)	162	<dl	23.82	51.76	<dl	304.86	172.47	n/t
59c	grey	162	<dl	42.42	84.76	114.8	436.07	359.91	n/t
59d	Orange glue	162	<dl	41.41	83.18	94.92	454.19	288.1	n/t
59e	Specimen	162	<dl	<dl	46.94	<dl	248.68	244.27	n/t
66d	Clean	140	<dl	<dl	<dl	<dl	731.73	196.46	1585.51

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
66e	Grey	140	<dl	<dl	<dl	<dl	1692.05	151.34	1944.09
72a	Clean	155	<dl	<dl	<dl	<dl	320.32	<dl	974.13
72b	Orange	155	<dl	<dl	<dl	<dl	171.68	55.16	722.77
72c	White	155	<dl	<dl	<dl	<dl	229.29	40.9	720.9
73a	Brown	111	<dl	49.1	134.41	<dl	773.24	274.86	1759.1
73b	Clean	111	44.5	40.09	106.58	<dl	211.53	253.42	1637.66
76e	Cream	204	188.61	26.81	50.34	3216.51	226.26	355.12	n/t
76f	Orange	204	3736.23	15.28	34.27	1668.23	1103.07	760.75	n/t
76g	Peach	204	62.62	25.65	57.78	5742.91	280.56	398.09	n/t
76h	Yellow	204	1060.57	22.03	76.4	5570.47	597.41	293.86	n/t
78a	Clean	142	<dl	7.5	57.98	<dl	744.07	506.14	n/t
78b	Grey	142	4.3	6.14	60.3	<dl	583.29	569.17	n/t
79a	Clean	139	<dl	<dl	<dl	<dl	434.75	273.31	678.49
79b	Yellow	139	<dl	<dl	<dl	<dl	769.28	251.44	496.47
Ac	Clean	124	16.44	<dl	7.64	<dl	365.76	563.71	n/t
Ad	Yellow	124	104.3	<dl	7.16	<dl	341.47	565.55	n/t
Air	n/a	n/a	<dl	<dl	<dl	<dl	<dl	<dl	0
Bu a01	n/a	103	<dl	39.42	<dl	<dl	846.02	613.11	207.48
Bu a02	n/a	103	<dl	<dl	80.49	<dl	452.23	383.2	275.34
Bu a03	n/a	103	<dl	36.5	<dl	<dl	949.42	606.6	292.23
Bu a04	n/a	103	<dl	34.66	<dl	<dl	555.53	396.31	314.56
Bu a05	n/a	103	<dl	37.48	<dl	<dl	978.83	821.75	348.54
Bu a06	n/a	103	<dl	34.76	<dl	<dl	1026.41	768.93	354.17
Bu a07	n/a	103	<dl	<dl	<dl	<dl	737.67	545.83	354.66
Bu a08	n/a	103	<dl	<dl	<dl	<dl	1079.13	684.27	357.18
Bu a09	n/a	103	<dl	36.41	<dl	<dl	774.66	594.85	368.35
Bu a10	n/a	103	<dl	<dl	<dl	<dl	388.16	411.65	375.73
Bu a11	n/a	103	<dl	36.99	<dl	<dl	750.87	998.93	384.95
Bu a12	n/a	103	<dl	37.67	<dl	<dl	879.71	660.97	387.38
Bu a13	n/a	103	<dl	34.76	<dl	<dl	825.15	673.01	421.26
Bu a14	n/a	103	<dl	38.45	<dl	<dl	761.46	657.67	464.17
Bu a15	n/a	103	<dl	36.31	<dl	<dl	918.45	544.08	479.22
Bu a16	n/a	103	<dl	32.43	<dl	<dl	854.27	775.53	489.42
Bu a17	n/a	103	<dl	39.22	<dl	<dl	1033.2	819.13	518.35
Bu a18	n/a	103	<dl	37.48	<dl	<dl	795.83	700	526.89
Bu a19	n/a	103	<dl	47.18	<dl	<dl	761.17	593.98	569.32
Bu a20	n/a	103	<dl	44.17	<dl	<dl	1112.62	716.21	577.67
Bu a21	n/a	103	<dl	<dl	105.92	<dl	1082.52	935.24	641.36
Bu a22	n/a	103	<dl	43.69	<dl	<dl	895.15	649.03	719.03
Bu a23	n/a	103	<dl	42.33	<dl	<dl	1058.74	617.77	963.3
Bu b01	n/a	103	<dl	<dl	<dl	<dl	1054.56	1336.12	353.2

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
Bu b02	n/a	103	<dl	30.97	<dl	<dl	999.03	1044.85	364.27
Bu b03	n/a	103	<dl	39.81	<dl	<dl	1002.52	965.24	370.58
Bu b04	n/a	103	<dl	<dl	<dl	<dl	1029.71	1117.86	391.07
Bu b05	n/a	103	<dl	37.67	<dl	<dl	1156.8	1186.8	392.62
Bu b06	n/a	103	<dl	42.04	<dl	<dl	1022.82	998.83	392.91
Bu b07	n/a	103	<dl	34.95	<dl	<dl	963.11	1142.91	394.56
Bu b08	n/a	103	<dl	39.81	<dl	<dl	1136.5	1272.82	405.92
Bu b09	n/a	103	<dl	32.04	<dl	<dl	1179.22	1217.18	410.78
Bu b10	n/a	103	<dl	38.16	<dl	<dl	1099.13	1127.09	411.36
Bu b11	n/a	103	<dl	<dl	93.59	<dl	1215.34	1748.06	416.12
Bu b12	n/a	103	<dl	33.01	<dl	<dl	1030	1114.08	422.82
Bu b13	n/a	103	<dl	<dl	112.72	<dl	912.43	1633.11	440.49
Bu b14	n/a	103	<dl	<dl	137.09	<dl	940.58	1498.83	442.23
Bu b15	n/a	103	<dl	33.01	<dl	<dl	1086.99	1410.19	443.3
Bu b16	n/a	103	<dl	44.76	<dl	<dl	1196.31	1343.3	447.77
Bu c04	Clean	103	9.69	54.91	97.75	<dl	1087.66	93.79	n/t
Bu c05	Grey	103	1794.95	47.48	103.82	<dl	405.87	154.91	n/t
Bu c06	White	103	1479.13	36.9	99.86	<dl	706.14	318.69	n/t
Bu c07	White	103	432.13	54.98	99.45	<dl	870.67	252.93	n/t
Ce	Brown	135	2.93	16.96	<dl	<dl	104.51	46.66	n/t
Cf	Clean	135	10.01	37.18	<dl	<dl	193.73	98.77	n/t
Cg	Cream	135	<dl	16.42	<dl	<dl	121.81	48.34	n/t
Ch	Grey	135	5.72	38.8	<dl	81.7	201.07	106.21	n/t
Dc	Clean	114	2.01	216.64	<dl	<dl	171.61	323.53	n/t
Dd	Yellow was Cream	114	<dl	518.52	<dl	<dl	655.21	417.78	n/t
Ed	Clean	112	<dl	<dl	73.9	<dl	827.65	292.86	n/t
Ee	Clean	112	<dl	<dl	68.76	<dl	770.11	261.05	n/t
Ef	Grey	112	<dl	<dl	73.72	<dl	861.94	333.21	n/t
F06c	Black	129	5516.05	<dl	26.12	<dl	56.55	1460.95	n/t
F06d	Cream	129	26.8	6.09	72.07	<dl	84.98	1096.05	n/t
F06e	Grey	129	58.26	12.33	94.89	<dl	93.3	1348.11	n/t
F06f	Peach	129	56.98	9.5	88.15	<dl	451.1	1583.48	n/t
F06g	Yellow	129	50.86	11.54	101.73	59.52	80.48	1190.63	n/t
F07d	Brown	163	166.56	20.12	<dl	<dl	147.12	5614.29	90.37
F07e	Brown 1	163	560.25	16.13	<dl	<dl	152.94	6066.81	97.24
F07f	Brown 2	163	154.29	20.18	<dl	<dl	112.02	6030.8	103.99
F07g	Brown 3	163	249.26	23.13	<dl	<dl	106.56	7071.1	174.23
F07h	Brown 4	163	278.28	17.79	<dl	<dl	124.66	7565.89	99.75
F07i	Brown 5	163	223.25	17.73	<dl	<dl	106.32	6992.76	104.36
F07j	Brown 6	163	170.43	16.87	<dl	<dl	130.49	6138.04	95.58

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
F07k	Clean 1	163	<dl	18.1	<dl	<dl	123.31	7085.58	93.37
F07l	Clean 2	163	18.71	24.23	<dl	<dl	112.02	5325.4	121.6
F07m	Clean 3	163	<dl	18.71	<dl	<dl	99.88	6807.55	113.56
F07n	Clean 4	163	<dl	19.51	<dl	<dl	114.11	6296.26	114.6
F07o	Clean 5	163	<dl	17.42	<dl	<dl	99.45	5928.47	100
F07p	Clean 6	163	<dl	22.27	<dl	<dl	84.05	6289.63	141.53
F07q	Cream 1	163	250.25	18.77	<dl	<dl	151.17	8325.28	141.66
F07r	Cream 2	163	153.25	13.44	<dl	<dl	134.29	6655.64	105.34
F07s	Cream 3	163	455.21	19.2	<dl	<dl	167.42	7473.74	133.31
F07t	Cream 4	163	134.72	21.29	<dl	<dl	127.55	5689.45	94.97
F07u	Cream 5	163	133.99	18.83	<dl	<dl	139.02	5954.97	107.3
F07v	Cream 6	163	156.44	23.31	<dl	<dl	123.5	5487.79	81.84
F07w	Grey	163	739.33	19.02	<dl	<dl	138.9	287.06	3529.69
F09c	Brown	127	95.43	15.04	<dl	<dl	<dl	223.54	810.94
F09d	Brown	127	<dl	<dl	<dl	<dl	<dl	154.88	1136.69
F09e	Clean	127	<dl	12.83	<dl	<dl	<dl	253.23	1322.44
F10c	Clean	74	<dl	40.81	103.59	<dl	269.14	674.05	n/t
F10d	White	74	<dl	34.01	114.62	97.8	457.4	626.8	n/t
F11e	Brown	64	3008.26	<dl	80.38	<dl	221.71	716.99	n/t
F11f	Clean	64	34.16	<dl	86.78	<dl	80.53	269.19	n/t
F11g	Cream	64	1593.84	<dl	86.48	<dl	278.64	462.91	n/t
F11h	Yellow	64	1611.48	<dl	81.05	<dl	145.05	344.73	n/t
F13d	Clean1	95	<dl	<dl	<dl	<dl	144	5939.79	138.74
F13e	Clean 2	95	<dl	17.05	<dl	<dl	193.47	6689.37	153.47
F13f	Clean 3	95	<dl	42.32	<dl	<dl	212.95	6993.05	163.79
F13g	Clean 4	95	36.74	<dl	<dl	<dl	229.58	6436.21	151.16
F13h	Clean 5	95	<dl	<dl	<dl	<dl	224.84	7219.37	246.42
F13i	Clean 6	95	<dl	<dl	47.47	<dl	148.84	8690.32	189.37
F13j	Grey	95	992.63	43.89	<dl	<dl	201.89	9816.63	228.42
F13k	Grey 1	95	1406	41.68	<dl	<dl	180.84	10562.74	197.16
F13l	Grey 2	95	1934.74	<dl	<dl	<dl	220.95	6797.89	147.26
F13m	Grey 3	95	1912.53	<dl	<dl	<dl	229.68	9452.53	158.11
F13n	Grey 4	95	2471.26	<dl	<dl	<dl	192.53	7965.26	137.16
F13o	Grey 5	95	1886.42	<dl	<dl	<dl	219.26	8122.53	114.63
F13p	Grey 6	95	1861.68	<dl	<dl	<dl	203.16	7546.63	187.47
F13q	White	95	42.32	<dl	<dl	<dl	159.68	5252.32	177.26
F14d	Clean	141	7.45	<dl	127.82	34644.51	71.65	1428.57	n/t
F14e	Cream	141	13.47	<dl	143.8	34367.37	78.53	1142.68	n/t
F14f	Orange	141	249.77	<dl	135.06	35534.41	<dl	<dl	n/t
F14g	Peach (missed the spot)	141	8.31	<dl	129.76	34893.28	74.37	1286.7	n/t

Table 13 (continued)

Sample	Fluorescence	Density g/m²	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Cl µg/g	S µg/g	Si µg/g
F16b	Clean	76	5.78	<dl	25.99	<dl	148.59	612.25	n/t
F16c	White	76	13.21	<dl	26.71	<dl	162.66	583.01	n/t
F17c	Clean	107	3.27	<dl	9.57	<dl	162.71	74.62	n/t
F17d	Yellow	107	9.39	4.04	6.06	<dl	178.48	82.38	n/t
F17e	Yellow	107	6.79	3.75	8.23	<dl	175.3	75.09	n/t
F18b	Clean	111	5.62	<dl	82.03	67.39	103.84	76.72	n/t
F18c	Yellow	111	20.82	<dl	90.99	70.98	166.29	47.07	n/t
F20f	Clean	147	3	<dl	8.86	1354.71	287.43	94.71	n/t
F20g	Clean	147	<dl	<dl	9.93	1445.43	364.64	179.5	n/t
F20h	Cream	147	2.47	<dl	8.22	1173.29	331.47	86.17	n/t
F20i	Cream	147	3.03	<dl	6.86	527.28	253.57	81.72	n/t
F21c	Clean	153	<dl	<dl	19.43	366.47	388.09	118.52	n/t
F21d	Specimen	153	<dl	<dl	<dl	<dl	131.68	302.28	n/t
F21e	Yellow	153	<dl	<dl	22.77	469.81	437.43	127.66	n/t
F22d	Clean	173	<dl	<dl	<dl	242.08	369.08	79.48	93.87
F22e	Grey	173	<dl	<dl	<dl	<dl	64.22	33.87	10071.85
F22f	Missed White spot	173	<dl	<dl	<dl	149.31	445.55	80.81	1664.91
F22g	Specimen	173	<dl	<dl	<dl	<dl	1232.95	1299.6	531.33
F24c	Clean	159	<dl	<dl	<dl	<dl	71.32	112.77	28.81
F24d	White	159	<dl	<dl	<dl	<dl	193.21	326.35	<dl
F26a	Black	164	5973.57	<dl	49.18	<dl	87.33	2112.06	n/t
F26b	Cream	164	523.75	4.81	29.6	<dl	330.87	1073.37	n/t
F26c	Cream Glue	164	55.72	5.99	43.15	51.74	89.79	703.08	n/t
F26d	Grey	164	329.53	6.8	53.1	<dl	70.29	1299.33	n/t
F26e	Grey (was Clean)	164	87.29	6.16	45.15	<dl	111.08	1456.4	n/t
F26f	Orange	164	3392.37	8.8	69.02	71.98	326.25	1609.8	n/t
Hb b	Sheet	165	<dl	<dl	48.89	<dl	5.77	3.75	n/t
Pc b	n/a	80	<dl	<dl	<dl	<dl	556.99	<dl	105.06
Pc c	n/a	80	<dl	<dl	<dl	<dl	575.9	77.83	113.13
Pc d	n/a	80	<dl	<dl	<dl	<dl	582.65	<dl	115.54
Pc e	n/a	80	<dl	<dl	<dl	<dl	624.7	86.75	111.33
Pc f	n/a	80	<dl	<dl	<dl	<dl	702.65	84.7	109.76
Pc g	n/a	80	<dl	<dl	<dl	<dl	666.27	<dl	119.28
Plastic	n/a	n/a	<dl	<dl	<dl	<dl	23.63	<dl	n/t
Type b	Folder	217	<dl	<dl	48.9	<dl	156.98	198.3	n/t

- Foxing refers to a possible foxing stain. This stain is sometimes visible by eye, which can indicate foxing. Foxing will flouresce under UV light

- n/t = not tested; dl = detection limit; n/a = not applicable

The results from the PIXE element line mapping are given in Figure 25.

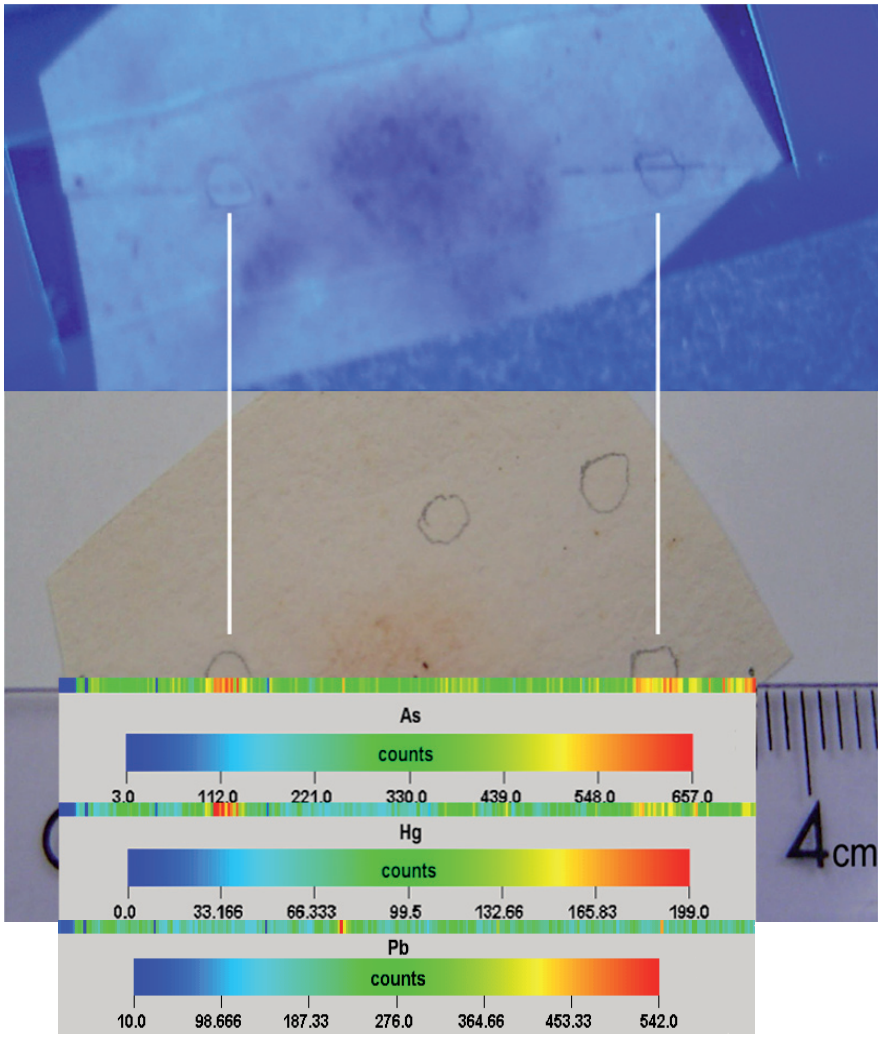

Figure 25 Distribution of arsenic, mercury and lead across a backing sheet from the third Earl of Bute collection (18th century). Element line mapping was carried out over the 4 cm distance between the two pencil dots, along the line indicated by the ruler. Concentrations are colour-coded with red being the most concentrated, and blue the least. The marked fluorescent areas (circled in pencil) correlate with the observed elevated concentrations in arsenic and mercury.

6.3 Mercury Speciation

A summary of the results for the XPS analysis is given in Table 14.

Table 14 Summary of XPS results indicating the presence and valence state of mercury in the samples analysed

Sample	Fluorescence	Instrument	Binding Energy (eV)	Valence State	Date specimen collected
19h	Clean	1,2,3	Not found		1930
19i	Yellow	1 2 3	101.0 (± 0.2) 101.6 (± 0.2)	+2	1930
19j	Orange	1 2	101.2 (± 0.2) 102.1 (± 0.2) 101.6 (± 0.2)	+2	1930
Ae	Clean	2 3	Not found Not found		1932
Af	Yellow	2	100.3 (± 0.2)	+1	1932
Ag	Peach	3	100.9 (± 0.2) 100.7 (± 0.2)	+1	1932
F14h	Clean	3	100.5 (± 0.2)	+1	1884
F14i	Brown	3	100.6 (± 0.2)	+1	1884

Standard Mercury Compounds

The binding energies for the Hg (4f 7/2) peak in the XPS spectra of the various standard mercury compounds are given in Table 15.

Table 15 Binding energies for the three valence states of mercury from analysis of standard compounds

Valence State	Binding Energy (eV)
+2	101.4 (± 0.2)
+1	100.8 (± 0.2)
0	99.5 (± 0.2)

The XPS spectra for the standard mercury compounds (both Hg(I) and Hg(II)), are shown in Figure 26.

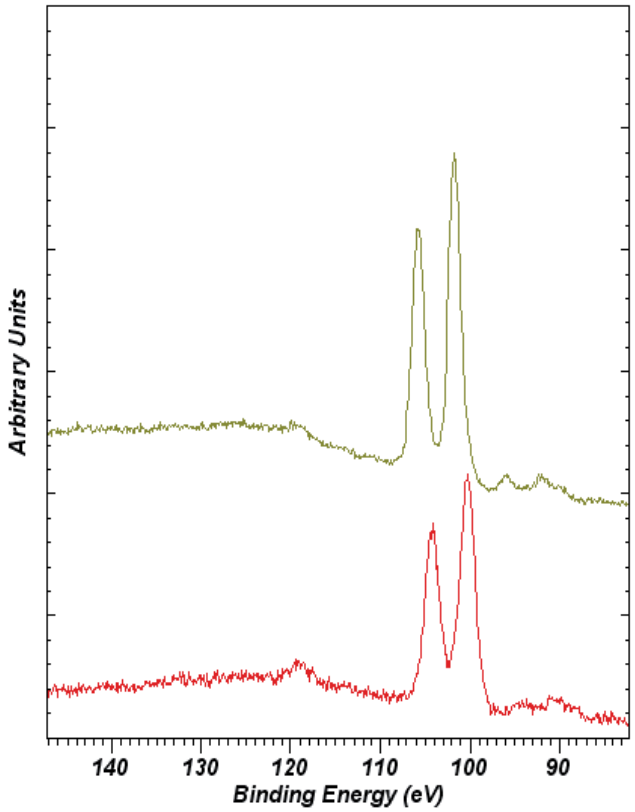

Figure 26 XPS spectra of standard compounds. Red is Hg (I) and Green is Hg (II)

Samples 19

The elements identified by XPS in samples 19h, 19i and 19j are given in Tables 16–18.

Table 16 Elements detected by XPS in sample 19h

Peak	Position BE (eV)	Peak Area (cps)	RSF	Concentration (mass %)
C (1s)	285	960517	12.011	65.76
O (1s)	532	856881	15.999	25.41
N (1s)	400	96379.	14.007	4.28
Ca (2p)	347	15631	40.078	0.53
S (2p)	168	10382	32.065	0.80
Cl (2p)	200	6998	35.460	0.45
Al (2p)	74	6768	0.193	1.55
Na (1s)	1076	5760	1.685	0.12
Si (2s)	153	7818	0.324	1.10

Table 17 Elements detected by XPS in sample 19i

Peak	Position BE (eV)	Peak Area (cps)	RSF	Concentration (mass %)
C 1s	285	845284	0.278	60.09
O 1s	532	720764	0.780	22.20
N 1s	400	254445	0.477	11.73
Hg 4f	101	65402	6.915	3.21
Cl 2p	198	20613	0.891	1.37
S 2p	168	7813	0.668	0.63
Al 2p	75	2127	0.193	0.51
Si 2s	154	1812	0.324	0.26

Table 18 Elements detected by XPS in sample 19j

Peak	Position BE (eV)	Peak Area (cps)	RSF*	Concentration (mass %)
C 1s	285	869789	0.278	63.47
O 1s	532	771654	0.780	24.39
N 1s	400.	115383	0.477	5.46
Hg 4f	101	58779	6.915	2.96
Cl 2p	200	12887	0.891	0.88
S 2p	169	3697	0.668	0.31
Al 2p	75	5078	0.193	1.24
Br 3p	184	5622	1.279	0.60
Si 2s	153	3780	0.324	0.56
Ca 2p	347	3135	1.833	0.11

***RSF** (Relative Sensitivity Factor) used for quantifying doublet peaks. Absolute ion peak areas have a higher variance of 59% as opposed to relative ion peak areas which vary by an average of 16%.

The analysis of samples 19 (h,i, and j) show a surface consisting of mainly carbon, oxygen, nitrogen, carbon and chlorine. There is also aluminium present (c. 1–2%) and possibly trace amounts of sulphur (<1%), although the low levels and other interfering peaks made assignment difficult.

Mercury was observed in both sample 19i (Figs. 27, 28), and 19j (Fig. 29) with surface concentrations of around 3% (30,000 ppm) unexpectedly high. The mercury doublet observed in the XPS spectrum from sample 19i (Fig. 29) is quite broad, average width 1.5 – 1.8 eV so much broader than seen for

other spectral peaks. There also seems to be some asymmetry to the peaks. This has been attributed to the presence of silicon, which would give some broadening to the Hg(4f) signal, thereby making peak assignment difficult. However, by accounting for the presence of silicon, and looking at the Hg(4d) peaks (BE ca. 360 eV), the mercury (with the Hg (4f $^{7}/_{2}$) peak at 101.0 and 101.6 in successive samples) is most probably present as Hg(II).

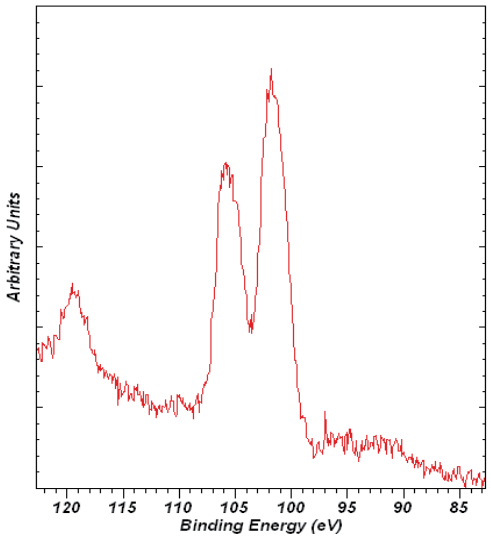

Figure 27 XPS spectra of sample 19i, showing asymmetric peaks of the mercury doublet.

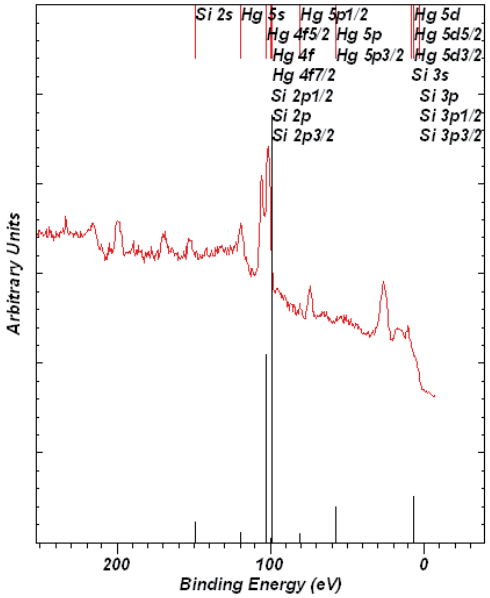

Figure 28 XPS spectra of sample 19i, showing where silicon could be affecting the peak width.

Sample 19j clearly contains mercury, as shown by the presence of the mercury doublet; the most intense peak is the Hg (4f 7/2) peak at 101.6 eV (Fig. 29).

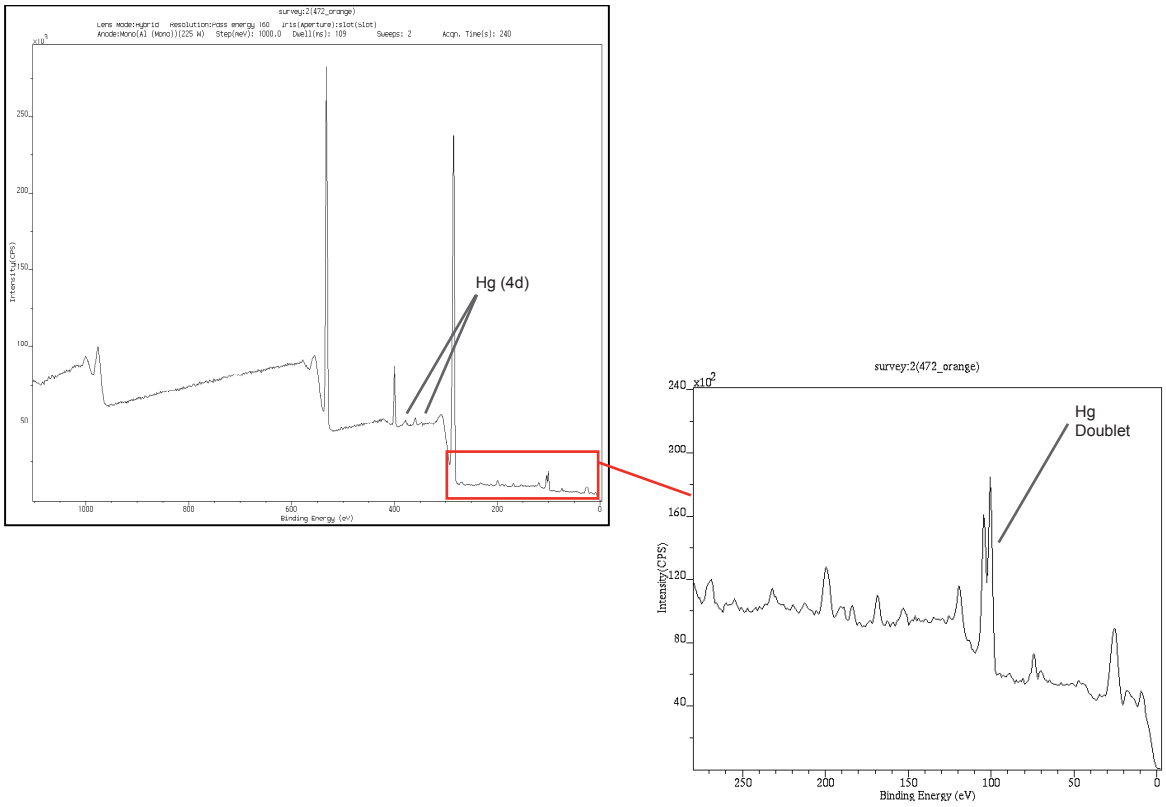

Figure 29 XPS spectrum of sample 19j, with an enlarged area showing the Hg(II) peak at 101.6 eV .

Figure 30 shows that two peaks are observed in the spectrum of sample 19h (bottom trace) at c.102 and c.119 eV, but no mercury doublet is evident (99–101eV). The two peaks have been attributed to traces of aluminium, although confirmation was difficult due to peak overlap.

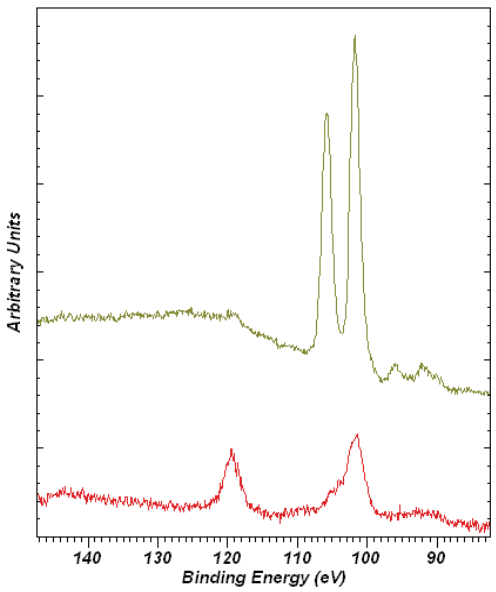

Figure 30 XPS spectra (80–150 eV) for samples 19h (bottom) and 19j (top). The mercury doublet is clearly visible in the 19j (top) spectrum.

Sample A

Tables 19-21 show the elemental results for each fluorescence of samples A. The XPS spectra for samples Ae and Af are given in Figure 27. The two spectra are very different. The sample Ae spectrum (bottom trace) shows two peaks at c.102 and c.119 eV, but no mercury doublet is evident (99–101 eV). The two peaks have been attributed to traces of aluminium, although confirmation was difficult due to peak overlap.

Table 19 Elements detected by XPS in sample Ae

Peak	Position BE (eV)	Peak Area (cps)	Concentration (At %)
O (1s)	532.61	5491.9	18.353
C (1s)	284.61	8476.9	79.483
Al (2p)	75.11	99.6	1.345
Cl (2p)	200.61	86.6	0.253
S (2p)	170.11	67.1	0.262
Si (2s)	102.61	38.1	0.303

Table 20 Elements detected by XPS in sample Ag

Peak	Position BE (eV)	Peak Area (cps)	Concentration (At %)
O 1s	532.71	6901.1	22.05
C 1s	284.71	8508.9	76.28
Al 2p	74.21	50.4	0.651
Hg 4f	100.71	1218.3	0.489
Cl 2p	198.71	123.2	0.345
S 2p	162.21	62.9	0.285

Table 21 Elements detected by XPS in sample Af

Peak	Position BE (eV)	Peak Area (cps)	Concentration (At %)
O 1s	532.495	454.5	40.69
C 1s	286. 495	222.6	58.39
N 1s	399. 495	2.742	0.4
Hg 4f	109. 495	14.9	0.21
Cl 2p	197. 495	2.725	0.31

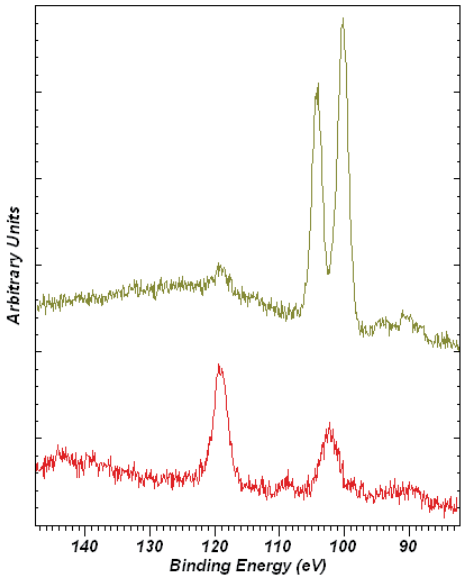

Figure 31 XPS spectra (80–150 eV) for sample Ae (bottom) and Af (top). The mercury doublet is clearly visible in the sample Af spectrum, but absent in the Ae spectrum.

The spectrum from sample Af (Fig. 31 top trace) clearly shows the mercury doublet; the most intense being the Hg (4f $^{7}/_{2}$) peak at 100.3 eV, which is characteristic of Hg(I).

Figure 32 shows the XPS spectrum of sample Ag, and indicates a considerable amount of mercury is present (0.439%). The near 1:1 ratio of chlorine to mercury suggests the presence of Hg_2Cl_2. The binding energy of the more intense peak of the mercury doublet (100.7 and 100.9 eV in the two samples analysed) indicates the presence of Hg (I).

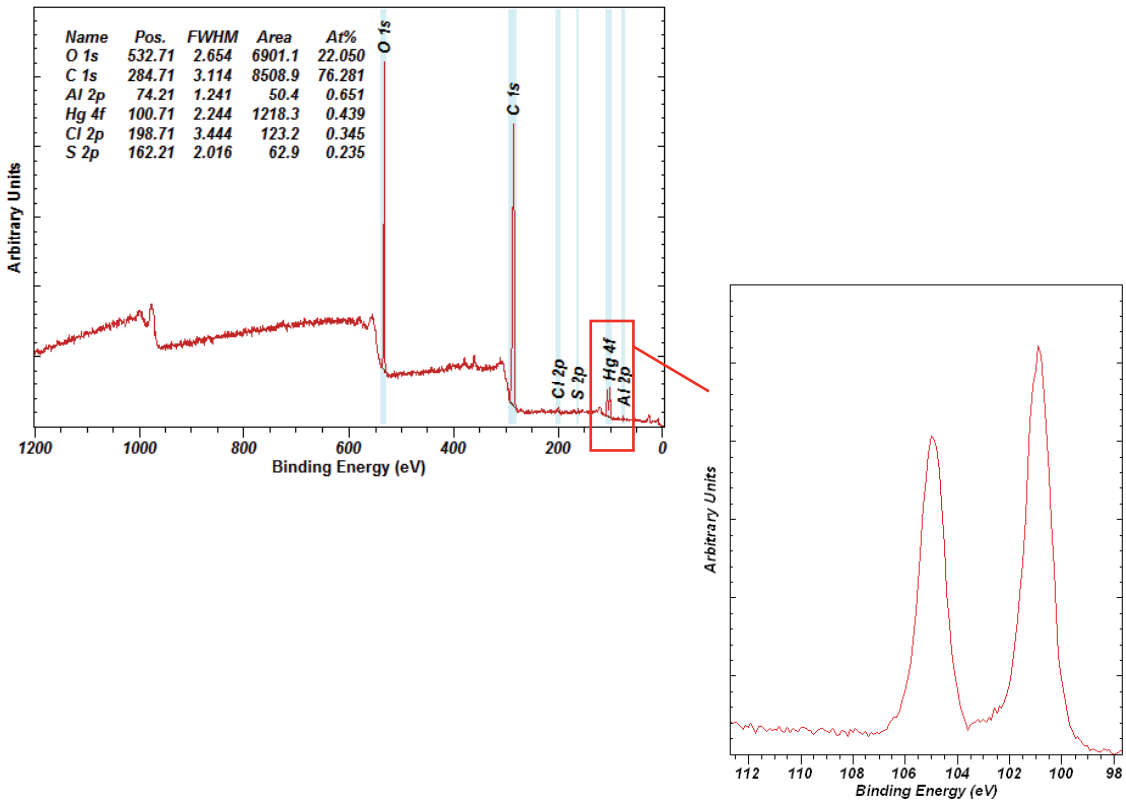

Figure 32 XPS spectrum of sample Ag, with an enlarged area showing the Hg(I) peak at 100.7 eV

Samples F14

Tables 22-23 show the elemental results for each fluorescence of samples F14.

Table 22 Elements detected by XPS in sample F14h

Peak	Position BE (eV)	Peak Area (cps)	Concentration (At %)
O (1s)	531.91	6150	17.678
C (1s)	284.91	9049	72.981
N (1s)	399.91	1796.8	8.446
Al (2p)	74.41	54.9	0.638
Hg 4f	100.41	790.7	0.256

Table 23 Elements detected by XPS in sample F14i

Peak	Position BE (eV)	Peak Area (cps)	Concentration (At %)
O 1s	532.21	4194.5	14.455
C 1s	284.71	7316.4	70.742
N (1s)	399.71	583.3	3.287
Hg 4f	100.71	15229.4	5.92
S 2p	162.21	1115.1	4.487
Cl 2p	198.21	367.8	1.110

From the survey spectrum of sample F14h (Fig. 33) it is evident that along with carbon and oxygen there is a significant contribution from nitrogen, aluminium and silicon-based species. The more intense peak of the mercury doublet (4f $^{7}/_{2}$) is asymmetrical, but the absence of asymmetry in the smaller peak (4f $^{5}/_{2}$) is indicative of an underlying Si (2p) photoelectron peak at *ca.* 102 eV, rather than a second mercury species. The binding energy of the Hg (4f $^{7}/_{2}$) is approximately 100.5 eV, indicating the presence of Hg(I).

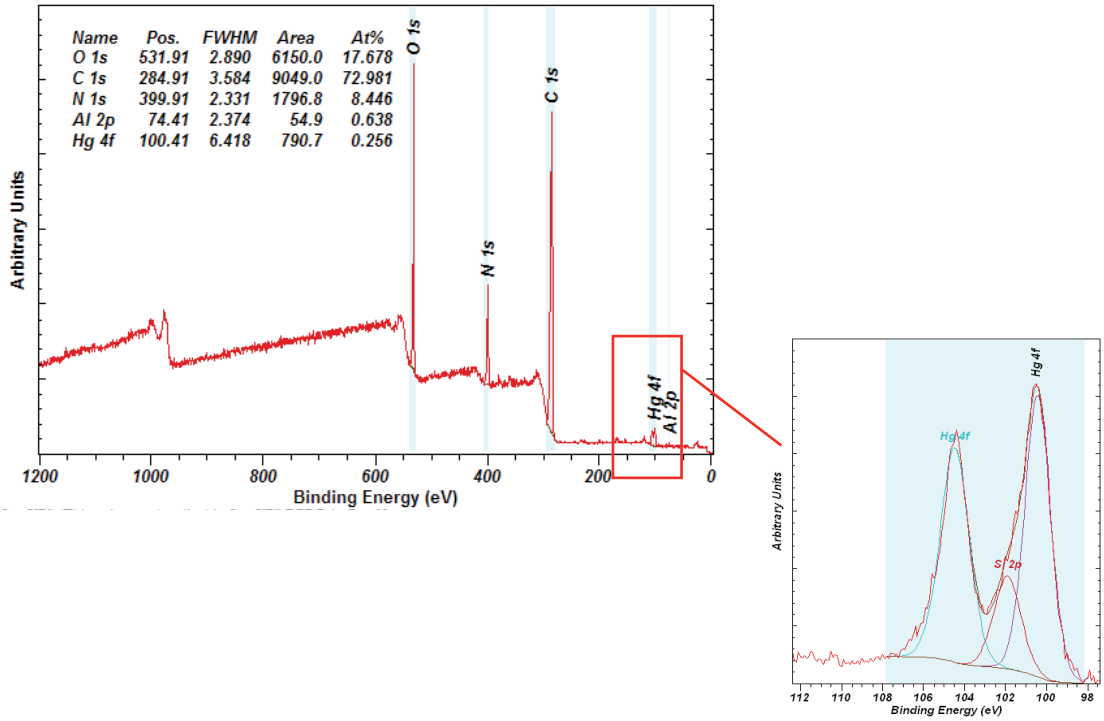

Figure 33 XPS spectrum for sample F14h, with an enlarged area showing the assymmetric mercury peak (4f 7/2)) due to the underlying Si(2p) peak.

The Hg (4f $^{7}/_{2}$) peak in the XPS spectrum of sample F14i (Fig. 34) is centred at 100.6 eV, indicating the presence of Hg(I). Mercury is present in a much higher concentration (5.92 at%) than seen in the corresponding 'clean' sample.

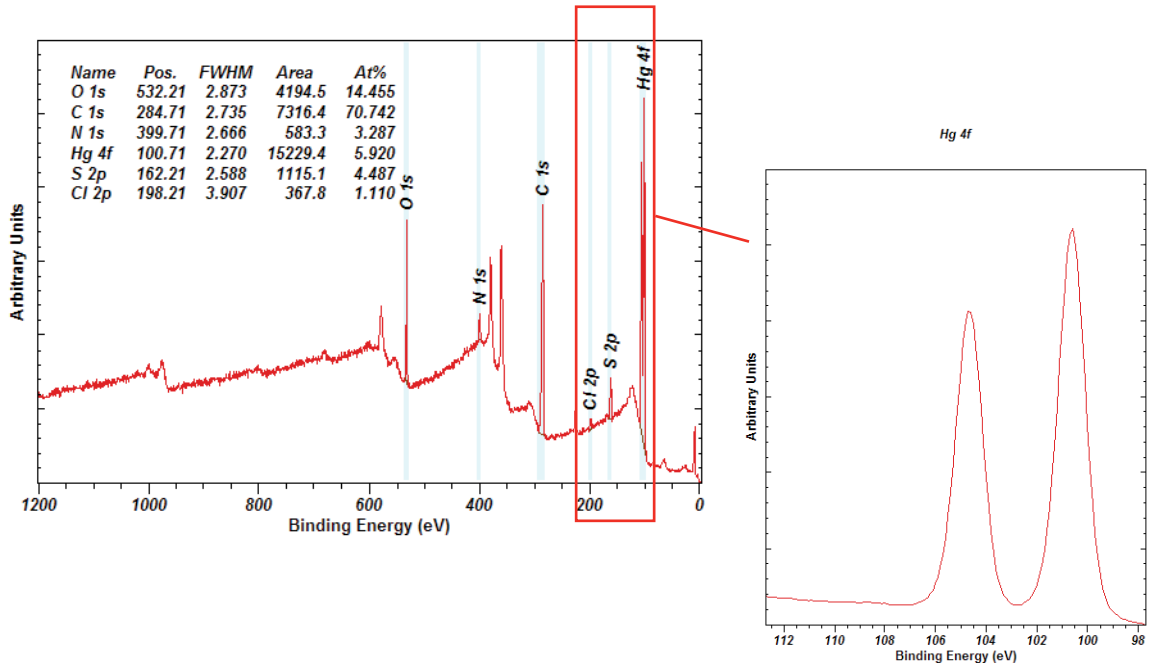

Figure 34 XPS spectrum of sample F14i, showing a significant concentration of Hg and the enlarged area showing Hg (4f7/2) peak centred at 100.6eV.

6.4 Accelerated Ageing

Observations made under UV light of the samples subjected to accelerated ageing tests, and control samples, are summarised in Table 24.

Table 24 Observed fluorescence on samples after accelerated ageing at 80°C with no RH control

Sample	Chemical Application	Aged	Observed Fluorescence
AA1a	Mercuric chloride	3 days	Cream
AA1b	Mercuric chloride	3 days	Cream
AA2a	Naphthalene	3 days	None
AA3a	Mercuric chloride and Naphthalene	3 days	Peach
AA4a	None	3 days	None
AA5a	Mercuric chloride	n/a	None
AA5b	Mercuric chloride	n/a	None
AA5c	Mercuric chloride	n/a	None
AA6a	Naphthalene	n/a	None
AA6b	Naphthalene	n/a	None
AA6c	Naphthalene	n/a	None
AA7a	Mercuric chloride and Naphthalene	n/a	None
AA7b	Mercuric chloride and Naphthalene	n/a	None
AA7c	Mercuric chloride and Naphthalene	n/a	None
AA8a	None	n/a	None
AA8b	None	n/a	None
AA8c	None	n/a	None

The IR spectra of four samples from the accelerated ageing tests are given in Figures 35–38. No significant differences are observed between the aged and non-aged paper, other than the presence of the added biocide.

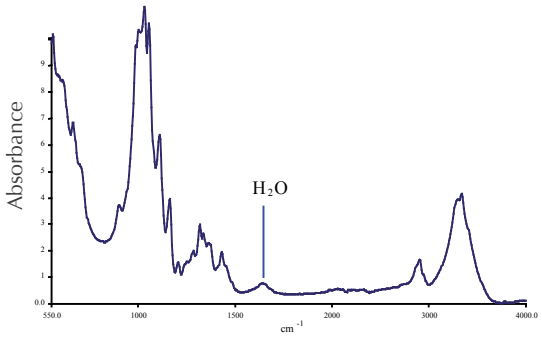

Figure 35 IR spectrum for the aged untreated filter paper, sample AA4

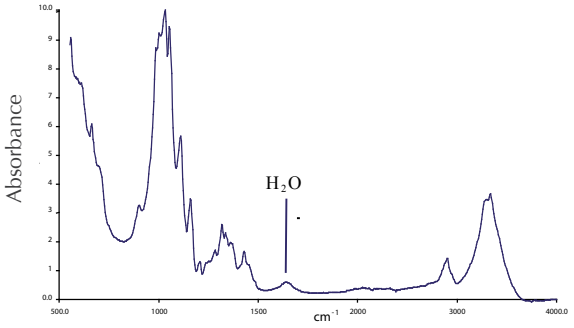

Figure 36 IR spectrum for the untreated filter paper, sample AA8

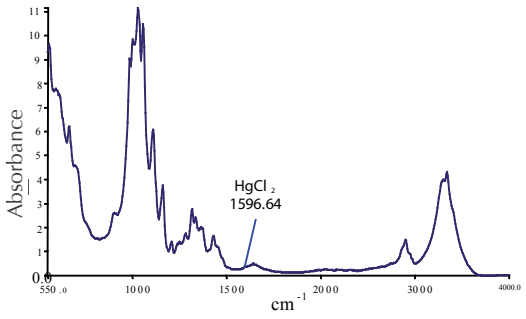

Figure 37 IR spectrum of AA1 showing the presence of mercuric chloride

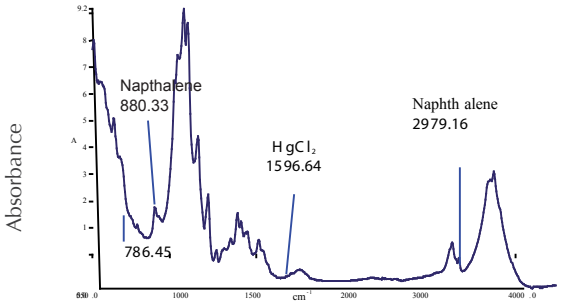

Figure 38 IR spectrum for sample AA3, showing the presence of both mercuric chloride and naphthalene

The results from the XPS analysis of the aged samples are summarised in Table 25.

Table 25 Valence states of the mercury in samples following accelerated ageing at 80°C with no RH control

Sample	Fluorescence	Applied Treatment	Binding Energy (eV)	Valence State
AA1a	Cream	$HgCl_2$	99.9 (± 0.2)	0/+1
AA1b	Cream	$HgCl_2$	100.8 (± 0.2)	+1
AA3a	Peach	$HgCl_2$ + naphthalene	100.8 (± 0.2)	+1
AA4a	None	None	Not found	n/a

The XPS spectrum for sample AA4 is given in Fig 39, and shows that the composition of the untreated filter paper consists of only carbon, oxygen and silicon. No mercury was observed. Small amounts of chlorine were present but at levels no greater than 1.5% (Fig 40).

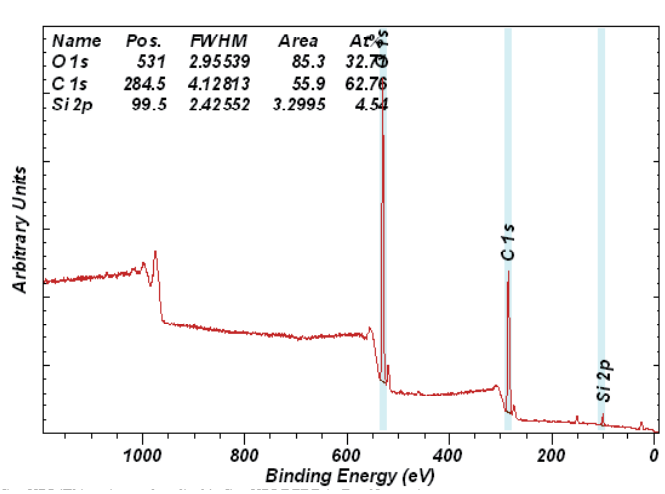

Figure 39 XPS spectrum for sample AA4, aged and untreated

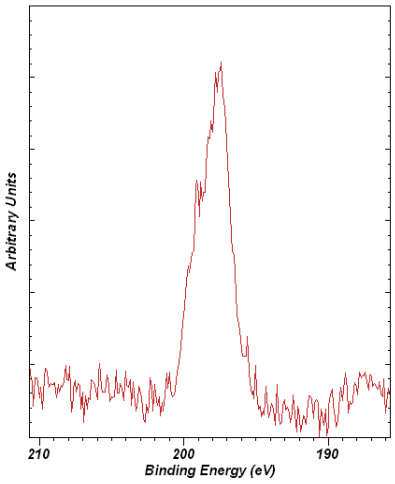

Figure 40 Spectrum for treated Whatman filter paper after accelerated ageing, AA1a, showing Cl(2p) peak.

The XPS survey spectrum for sample AA1a shows the presence of mercury (0.03%).

The binding energy of the Hg(4f $^{7}/_{2}$) peak is 99.9 eV (+/- 0.2 eV) and is therefore attributed to the $^{+}1$ state as the standard Hg_2I_2 gave a binding energy of ~100 eV. This is strengthened by the observation that the chlorine is still present (Fig. 41). Otherwise, it could have been attributed to the metallic state, the binding energy of metallic Hg being ~99.5 eV.

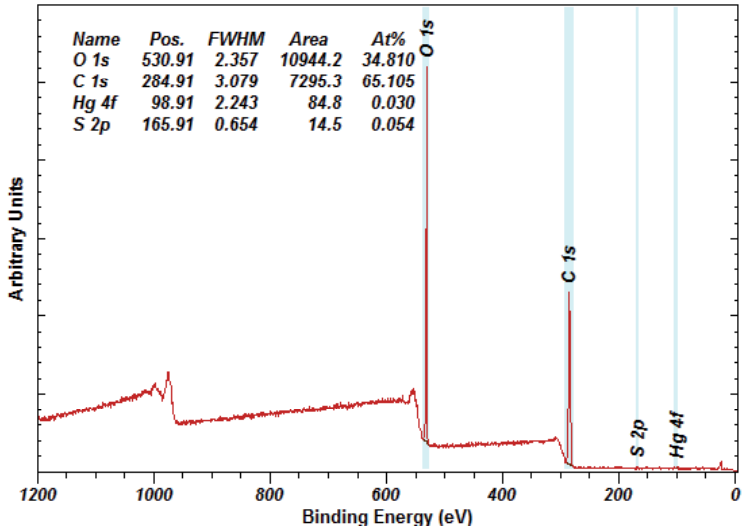

Figure 41 XPS survey spectrum for sample AA1a, treated with mercuric chloride and subjected to accelerated ageing. The observed sample fluorescence is cream.

Sample AA1b has the same concentration of mercury as the clean sample, however sulphur has also been detected and very low levels of chlorine. The binding energy of the mercury was 100.9 eV, and is comparable to Hg_2Cl_2 (Hg $^{+}$I). The chorine has most likely come from the mercuric chloride used in the accelerated aged samples. The sulphur may have come from various sources (e.g. heating source, handling/storage, atmosphere, the paper making process etc.) and may be expected by the high affinity of mercury for sulphur. The concentration of the mercury in both clean and cream is very similar and very low. The cream sample had a high concentration of mercuric chloride applied directly to the sheet

and so it is surprising that such a low concentration of both mercury and chlorine is present. It is possible that the results have been duplicated for the clean sample or that the area of fluorescence was not actually analysed. Past results have shown a definite inhomogenous distribution of mercuric chloride over a paper substrate; this is often why only small areas over the sheet fluoresce. It is therefore possible that the exact fluorescing area was not actually scanned by XPS.

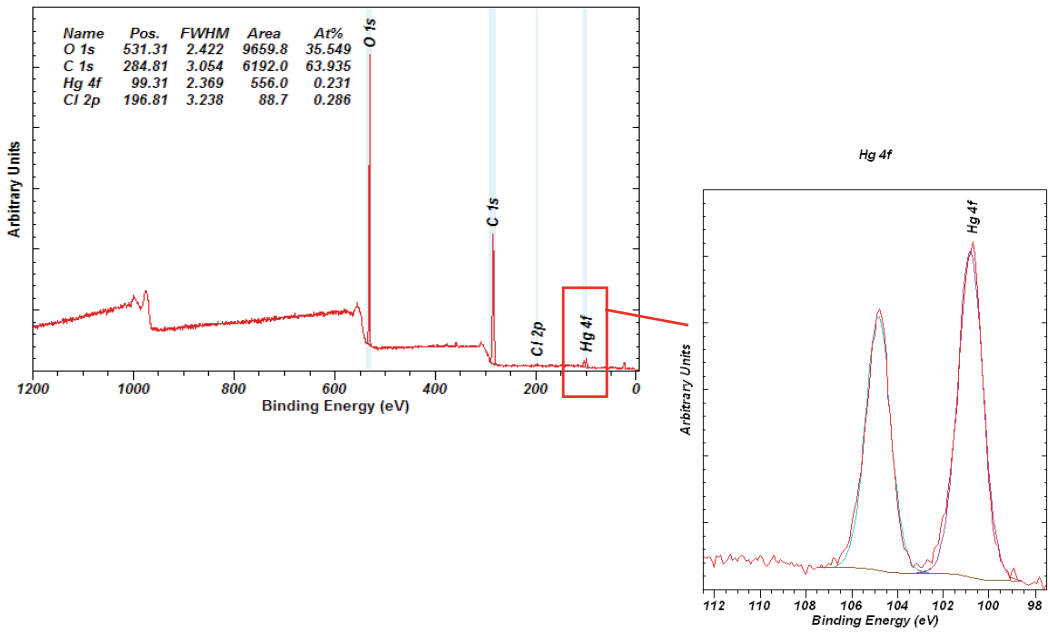

Figure 42 Spectrum for accelerated aged sample fluorescing peach AA3. Enlarged area of spectrum is showing mercury doublet.

The peach coloured fluorescence displayed much higher concentrations of mercury (c.8 times higher), the binding energy of which was 100.8 eV, which was the same as that observed for the cream sample (Hg^{+1}) (Fig. 41).

Unlike the cream sample, no sulphur was observed, but chlorine was found with a binding energy of 198.4 eV, again suggesting chloride. The Hg:Cl ratio was found to be 0.9:1 suggesting that the Hg and Cl are possibly present as Hg_2Cl_2 again supporting that the mercury is in the $^{+}1$ state.

The mercury binding energy changes on the accelerated aged samples to ~99.5 eV. This is characteristic of 'elemental' mercury, whereas samples not artificially aged have a higher binding energy, by some 1 to 1.5 eV. This would suggest that mercury is bound to a more electron withdrawing group during natural ageing.

6.5 Laser Ablation Decontamination

Tables 26-27 show the results of applying laser ablation to a section of a Bute herbarium sheet (Figs. 43 and 44). The areas treated were from the paper edge. Figure 44 shows an area of the herbarium sheet that has been treated with a laser. The sample is slightly lighter in colour after cleaning and so it was thought that it would be of interest to see if any of the heavy metals had been removed after laser ablation.

The difference in intensity between the dark area is shown clearly in Fig 43. Fig 44 is a slightly different area of the sheet but this does still indicate the effectiveness of the laser ablation to visibly reduce the dark staining.

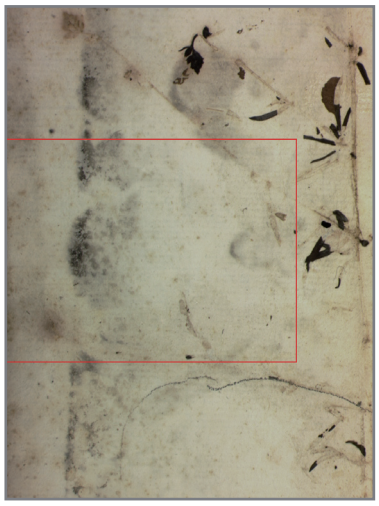

Figure 43 Red square is high-lighting the characteristic dark staining of a metal sulphide on a herbarium specimen sheet

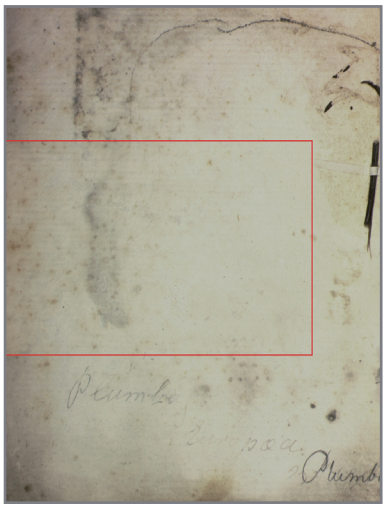

Figure 44 Red area is showing an area of the same paper that has been cleaned using laser ablation.

Table 26 Results of Pixe analysis before Laser ablation

Sample	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Si µg/g	S µg/g	Cl µg/g
Bu b01	0.010	<dl	<dl	<dl	<dl	353.20	1336.12	1054.56
Bu b02	0.010	<dl	30.97	<dl	<dl	364.27	1044.85	999.03
Bu b03	0.010	<dl	39.81	<dl	<dl	370.58	965.24	1002.52
Bu b04	0.010	<dl	<dl	<dl	<dl	391.07	1117.86	1029.71
Bu b05	0.010	<dl	37.67	<dl	<dl	392.62	1186.80	1156.80
Bu b06	0.010	<dl	42.04	<dl	<dl	392.91	998.83	1022.82
Bu b07	0.010	<dl	34.95	<dl	<dl	394.56	1142.91	963.11
Bu b08	0.010	<dl	39.81	<dl	<dl	405.92	1272.82	1136.50
Bu b09	0.010	<dl	32.04	<dl	<dl	410.78	1217.18	1179.22
Bu b10	0.010	<dl	38.16	<dl	<dl	411.36	1127.09	1099.13
Bu b11	0.010	<dl	<dl	93.59	<dl	416.12	1748.06	1215.34
Bu b12	0.010	<dl	33.01	<dl	<dl	422.82	1114.08	1030.00
Bu b13	0.010	<dl	<dl	112.72	<dl	440.49	1633.11	912.43
Bu b14	0.010	<dl	<dl	137.09	<dl	442.23	1498.83	940.58
Bu b15	0.010	<dl	33.01	<dl	<dl	443.30	1410.19	1086.99
Bu b16	0.010	<dl	44.76	<dl	<dl	447.77	1343.30	1196.31
Average values		0.00	25.39	21.46	0.00	406.25	1259.83	1064.07

Table 27 Results of PIXE analysis after Laser ablation

Sample	Wt (g)	Hg µg/g	As µg/g	Pb µg/g	Ba µg/g	Si µg/g	S µg/g	Cl µg/g
Bua1	0.01	<dl	39.42	<dl	<dl	207.48	613.11	846.02
Bua2	0.01	<dl	<dl	80.49	<dl	275.34	383.20	452.23
Bua3	0.01	<dl	36.50	<dl	<dl	292.23	606.60	949.42
Bua4	0.01	<dl	34.66	<dl	<dl	314.56	396.31	555.53
Bua5	0.01	<dl	37.48	<dl	<dl	348.54	821.75	978.83
Bua6	0.01	<dl	34.76	<dl	<dl	354.17	768.93	1026.41
Bua7	0.01	<dl	<dl	<dl	<dl	354.66	545.83	737.67
Bua8	0.01	<dl	<dl	<dl	<dl	357.18	684.27	1079.13
Bua9	0.01	<dl	36.41	<dl	<dl	368.35	594.85	774.66
Bua10	0.01	<dl	<dl	<dl	<dl	375.73	411.65	388.16
Bua11	0.01	<dl	36.99	<dl	<dl	384.95	998.93	750.87
Bua12	0.01	<dl	37.67	<dl	<dl	387.38	660.97	879.71
Bua13	0.01	<dl	34.76	<dl	<dl	421.26	673.01	825.15
Bua14	0.01	<dl	38.45	<dl	<dl	464.17	657.67	761.46
Bua15	0.01	<dl	36.31	<dl	<dl	479.22	544.08	918.45
Bua16	0.01	<dl	32.43	<dl	<dl	489.42	775.53	854.27
Bua17	0.01	<dl	39.22	<dl	<dl	518.35	819.13	1033.20
Bua18	0.01	<dl	37.48	<dl	<dl	526.89	700.00	795.83
Bua19	0.01	<dl	47.18	<dl	<dl	569.32	593.98	761.17
Bua20	0.01	<dl	44.17	<dl	<dl	577.67	716.21	1112.62
Bua21	0.01	<dl	<dl	105.92	<dl	641.36	935.24	1082.52
Bua22	0.01	<dl	43.69	<dl	<dl	719.03	649.03	895.15
Bua23	0.01	<dl	42.33	<dl	<dl	963.30	617.77	1058.74
Average values		0.00	38.33	93.20	0.00	451.76	659.48	848.57
Average values un-cleaned minus cleaned		0.00	-4.61	13.36	0.00	-45.51	600.35	215.49

- dl = detection limit

7 Discussion

7.1 Fluorescence as an Indicator of Biocide Residue

The biocide-treated sheets, observed under UV-A light, clearly showed areas of fluorescence that distinctly changed the colour of the paper: a single sheet typically gave rise to 4 or 5 different fluorescence colours. This fluorescence is broadly observed across the NMW collection, and is also evident in external British and foreign collections.

Although the fluorescent areas have the appearance of an aqueous application, with distinctly drop-like characteristics, they were clearly not the result of water droplets: the fluorescence was neither limited to the wet/dry interface, nor a blue/white in colour, as is often observed with water on cellulose. Furthermore, it is not likely that water was routinely applied to the sheets, as this would have been highly deleterious to the specimen - a rise in relative humidity (RH) encourages mould growth and possible insect infestation. There have been, however, numerous pesticide and fungicide applications made to herbarium collections since the17th century, most of which were applied in solution form. It is likely, therefore, that the fluorescence is associated with the presence of biocide residues from past treatments. From the literature, it is apparent that aqueous biocide applications frequently contained heavy metals, most typically mercury, arsenic and lead. Elemental line-mapping by PIXE (Fig. 45) confirmed that the observed fluorescence was, at least in part, related to the biocide treatment, as high levels of mercury and arsenic were observed only within the fluorescing areas. The specimen sheet analysed was from John Stuart, third Earl of Bute's herbarium (1713-1792). The specimens within this collection are quite likely to have been treated with both arsenic and mercury in a single treatment, as was common practice at the time.

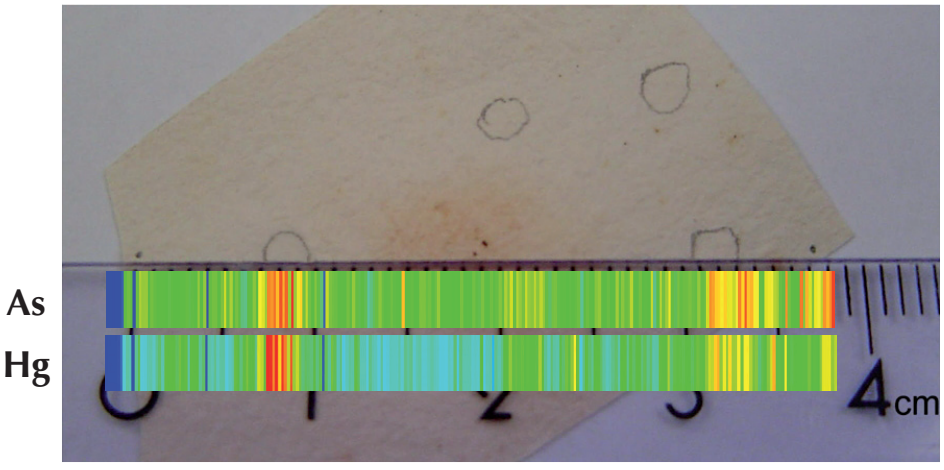

Figure 45 Distribution of arsenic and mercury across a specimen sheet from third Earl of Bute's herbarium (18th century). The fluorescent areas are marked with a pencil. The relative metal concentrations are colour-coded: red denotes the highest concentration, followed by yellow and then green. Blue denotes the lowest concentration.

There was little evidence, however, from the multi-elemental data to corroborate the PIXE elemental line-mapping. Comparison of the frequency distributions of heavy metal composition for both the coloured (105 samples, 8 observed colours, including 5 fluorescent) and non-coloured areas (74

samples), as determined by AAS, gave little indication that the heavy metal composition of the coloured areas was in any way different to the non-coloured areas. As is evident from Figure 46, the distribution patterns for mercury, arsenic and lead appeared similar for both sample sets.

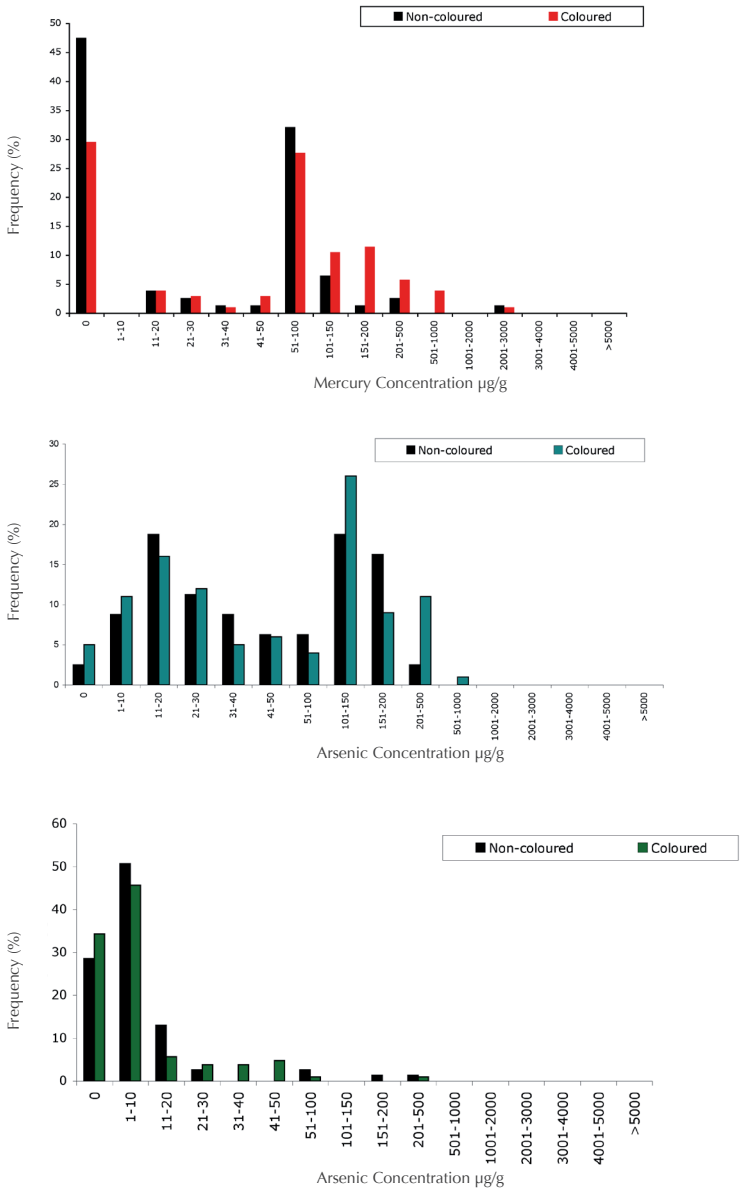

Figure 46 Frequency distributions of mercury, arsenic and lead content in both the coloured (n=105) and non-coloured (n=74) areas, following analysis by AAS.

Following PIXE analysis, however, the frequency distributions of heavy metal composition indicated that the observed coloured and/or fluorescent areas are likely to be associated with the presence of mercury (Fig.47). Although there is some overlap between the distributions of the coloured (139 samples) and non-coloured (117 samples) areas, 58% of the coloured samples contained mercury concentrations significantly higher than those observed in the non-coloured areas (samples containing [Hg] > 20µg/g).

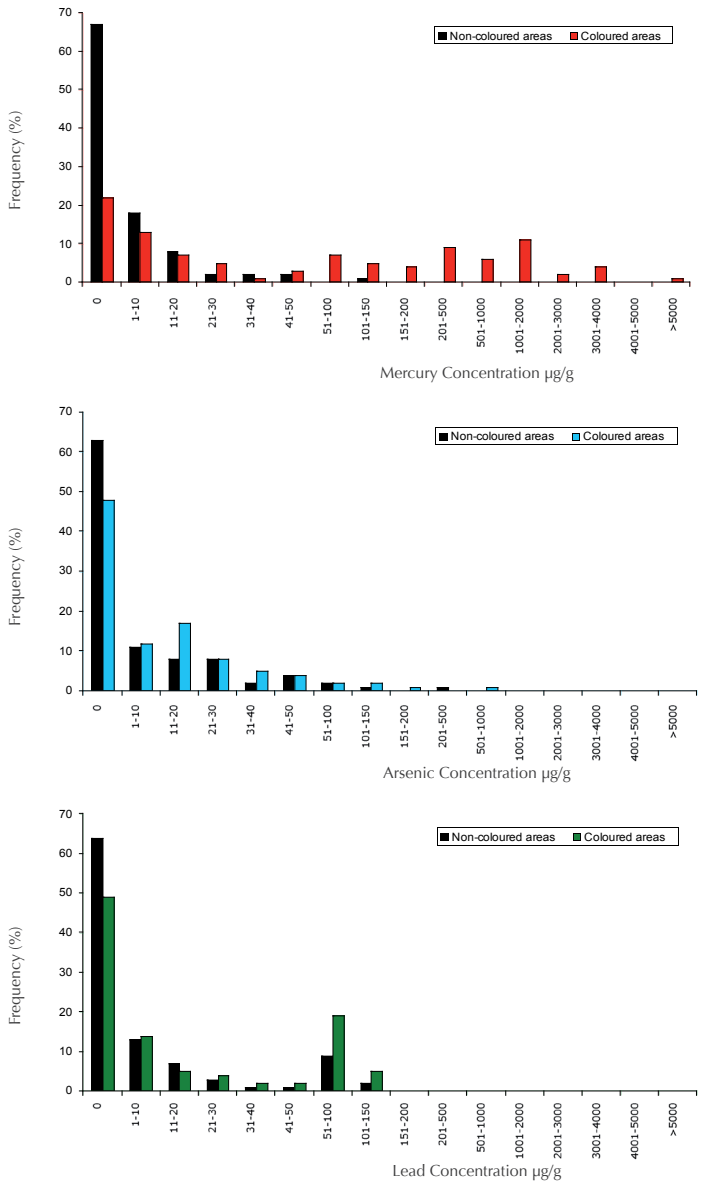

Figure 47 Frequency distributions of mercury, arsenic and lead content in both the coloured (n=139) and non-coloured (n=119) areas, following analysis by PIXE.

There was no significant difference observed in the arsenic and lead concentration distributions between the coloured and non-coloured areas. The presence of arsenic and lead, therefore, is not likely to have contributed to the development of the coloured and/or fluorescent areas on the specimen sheets.

The difference in results from the AAS and PIXE analyses may be empirical in nature. The samples were aggressively dissolved prior to the AAS analysis and volatile species (e.g. mercury and arsenic) may not have been analysed quantitatively. The PIXE analysis, on the other hand, was only micro-destructive, and required no sample preparation. Volatility should not, therefore, have been an issue.

The accelerated ageing tests, using mercury (II) chloride as the biocide treatment, confirmed that the observed fluorescence was directly associated with the mercury content. After three days of ageing, fluorescent areas had developed on the samples treated with mercury (II) chloride (Table 28).

The mercury (II) chloride treatment (samples AA1) gave rise to a cream-coloured fluorescence. No fluorescence was observed under UV-A light on any of the prepared samples prior to accelerated ageing.

Since many of the herbarium sheets within the NMW collection have also been treated with naphthalene, some of the accelerated ageing tests were carried out in the presence of naphthalene. Although naphthalene alone did not cause the development of fluorescent areas, when applied to the samples with mercury (II) chloride (sample AA3), a peach-coloured fluorescence was produced. Interestingly, one year after the accelerated ageing tests were complete, the fluorescence produced by the mercury (II) chloride treatment alone, on the AA1 samples, had turned from cream to peach in colour. The naphthalene may, therefore, act as a catalyst in the fluorescence development.

Table 28 Observed fluorescence on samples after accelerated ageing at 80°C with no RH control

Sample	Chemical Application	Aged	Observed Fluorescence
AA1a	Mercury (II) chloride	3 days	Cream
		1 year*	Peach
AA1b	Mercury (II) chloride	3 days	Cream
		1 year*	Peach
AA2a	Naphthalene	3 days	None
AA3a	Mercury (II) chloride and Naphthalene	3 days	Peach
AA4a	None	3 days	None

*samples were left for a year after 3 days of accelerated ageing

It is difficult to determine the length of natural ageing required to observe the development of the fluorescence after a biocide application. Several attempts have been made to relate artificial ageing to the equivalent time of natural ageing (Wilson, Harvey *et al*., 1955, Browning and Wink, 1968, Baer and Indictor, 1977, Zou, Uesaka *et al*., 1996), although Ströfer-Hua has expressed doubt that accelerated ageing methods can be used at all to predict the behaviour of certain papers (Ströfer-Hua, 1990).

The accelerated ageing tests were run for 3 days. This would usually be considered equivalent to approximately 30 years. However, the dry ageing employed (with no RH control) may well have increased the projected age in years. Furthermore, due to the amount of filter paper required for the standard accelerated ageing tests (ASTM/ISR research paper (1-14), the samples were rolled up tightly and twisted, putting the areas along the twisted margins of the paper under stress. The physical damage thus caused to the paper, breaking the glycosidic linkages, will have further accelerated localised cellulose degradation (Tímár-Balázsy and Eastop, 1998). Only these damaged areas developed the coloured fluorescence. The physical stress to the paper clearly contributed to the cellulose degradation and further aged the samples. Naturally aged paper will degrade through handling, exposure to light

and/or environmental pollution (Arnold, 2003, May and Jones, 2006).

It is also possible that the development of the fluorescence was impeded by the interaction between the metal ions in the biocide and the size and finish present on the specimen sheets. Although the migration of metal ions into paper from iron gall ink is directly proportional to the time elapsed since application (McNeil, 1984), the depth of penetration is dependent upon the amount and composition of application, how well the paper was sized and finished, and environmental factors (Reißland, 2002) .

Empirical observations would indicate that a minimum of 30 years is likely to be a good estimate of the length of natural ageing required to observe the development of the fluorescence after a biocide application, consistent with the accelerated ageing tests – the youngest specimen sheet in the NMW collection to display fluorescence is 30 years old (Table 8, specimen 22).

Some difficulties were experienced whilst carrying out the accelerated ageing process. To successfully age material spiked with chemicals, the seals on the vials must be hermetic. Due to the vapour pressure exerted by the chemicals during ageing, this proved hard to achieve: initially, the sub-standard seals were broken in each experiment and needed to be repeated. Eventually the lids were very carefully re-sealed during the ageing process and positive results were achieved. It is recommended, however, that Pyrex™ vials should be used for further research, fitted with a screw cap made of Bakelite™ and a Teflon™ packing.

The elemental analyses and the accelerated ageing studies have shown quite conclusively that fluorescence is produced over time when mercury (II) chloride is applied to a cellulose-based matrix. The ageing studies have also shown that the process may be catalysed by naphthalene. It is apparent that this fluorescence only develops when the cellulose begins to degrade, whether through oxidation or through mechanical stress, both of which result in chemical changes.

7.2 Differentiating the Eight Colours Observed on the Specimen Sheets

The UV-A survey of the collection identified eight different colours on the specimen sheets – five different fluorescent colours: white, cream, yellow, peach and orange; and a further three colours that did not fluoresce: black, grey and brown. It was not clear whether all of these observations were a result of historic mercury (II) chloride applications.

The accelerated ageing tests showed that the application of mercury (II) chloride to paper results in a cream-coloured fluorescence, which over time turns to peach. The various fluorescent colours may, therefore, be an artefact of time, may reflect variation in concentration, the degree of degradation, or be due to the presence of other compounds.

It is likely that the pesticide was applied to the botanical specimen by brush, leaving small fluorescent drip marks of higher pesticide concentration, as it was applied. The metal ions would have become rapidly enmeshed within the cellulose matrix and prevented from being distributed evenly across the paper surface. It was apparent from the PIXE elemental-mapping (Fig.45) that the mercury and arsenic biocide applications were unevenly distributed across the sheets, suggesting that inhomogeneity would be a key factor for consideration. In order to determine the extent of the inhomogeneity within the sampled areas, 6 replicate analyses were carried out from within the same small pencil-marked sample area (sampling diameter approx. 2–3 mm. See Fig.45). Table 29 shows the relative standard deviations from mean elemental composition for 12 different samples (n=6). The inhomogeneity within a single sampling area was large, with variations ranging from ±18–245% for mercury and ±14–245% for arsenic. Similar inhomogeneity was observed in elemental composition within the nine UV-A identifiable groups (including the non-coloured group). From Table 30, it is evident that the inhomogeneity across a colour group is comparable to that observed across a single sample, with relative standard deviations of up to ±318% for mercury, ±302% for arsenic, ±204% for lead and ±787% for barium. With such vast variation within groups, it is unlikely that multivariate statistical techniques (e.g. principal component analysis and cluster analysis) would be able to identify differences in elemental profiles between the various colours observed.

Table 29 Variation in mean elemental composition within small sample areas (n=6) of 12 samples. The high relative standard deviations illustrate the inhomogeneity of the elemental distribution. A sample of new photocopy paper was used as a control.

Sample Sheet	Area colour	Relative Standard Deviation (%)						
		Hg	As	Pb	Ba	S	Cl	Si
39	Non-coloured	–	113	–	–	6	4	9
42	Non-coloured	–	155	–	–	22	27	29
52	Non-coloured	155	245	–	8	10	3	4
F07	Non-coloured	245	13	–	–	10	13	15
F13	Non-coloured	245	175	245	–	13	20	23
42	Foxing	–	–	–	–	42	42	43
47	Foxing	–	–	–	–	8	8	6
39	White	–	49	–	–	11	12	16
52	Yellow	76	–	–	10	33	9	7
F07	Cream	59	17	–	–	17	12	21
F07	Brown	54	14	–	–	10	15	27
F13	Grey	18	245	–	–	16	9	20
Photocopy paper	Control	–	–	–	–	9	6	4

Table 30 Variation in elemental composition across the 9 identified groups of coloured and non-coloured areas on specimen sheets.

Area colour	n	Relative Standard Deviation (%)						
		Hg	As	Pb	Ba	S	Cl	Si
Non-coloured	119	278	227	199	787	202	73	92
Black	2	6	–	43	–	26	30	–
Brown	19	253	139	204	–	120	227	140
Grey	31	160	154	152	442	157	104	159
White	19	318	146	155	325	199	54	138
Cream	24	243	104	174	408	138	64	21
Yellow	22	140	302	151	247	67	48	138
Peach	14	180	157	127	264	102	69	374
Orange	8	85	127	101	245	96	75	283

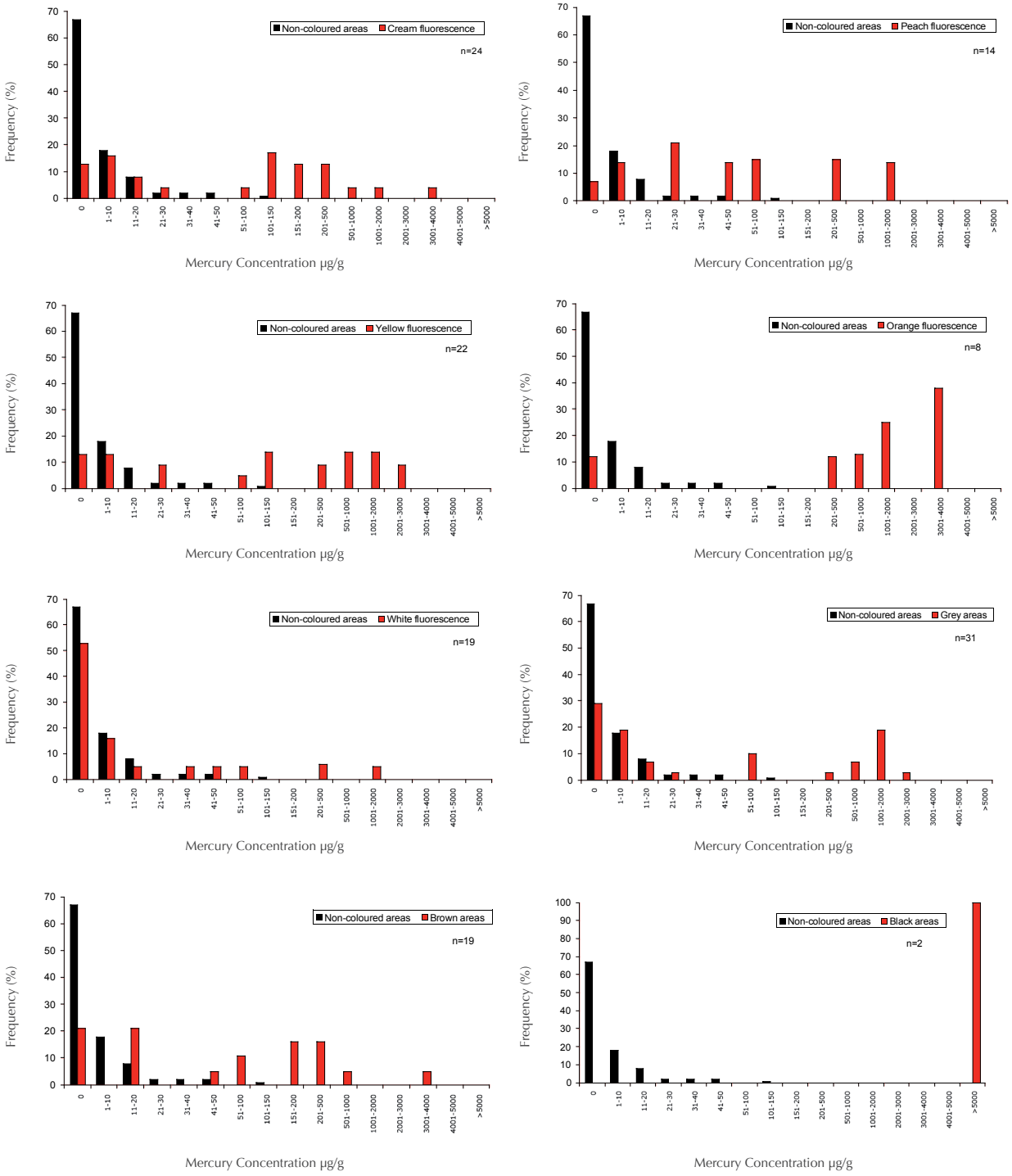

Figure 48 Frequency distributions of mercury content within the eight different coloured areas identified under UV-A light. Comparison is made with the frequency distribution observed for 119 non-coloured areas.

In order to establish whether there were any significant associations between the development of colour on the specimen sheets (fluorescent or otherwise) and the presence of toxic metals, elemental frequency distributions (mercury, arsenic and lead) for each coloured group were compared to those of the non-coloured group (Fig.48). Consideration of the mercury frequency distribution within the eight identified coloured groups has shown that there are some differences between the colour groups, although the inhomogeneity makes this difficult to quantify. Table 31 shows the percentage of samples within each colour group with significantly higher mercury content than the non-coloured areas (defined as [Hg] > 20µg/g).

Table 31 Percentage of samples within a colour group containing mercury concentrations significantly higher than those observed in the non-coloured areas ([Hg] > 20µg/g).

Colour Group	n	Samples with [Hg] > 20µg/g (%)
Cream	24	63
Peach	14	79
Yellow	22	74
Orange	8	88
White	19	26
Grey	31	45
Brown	19	58
Black	2	100
Non-coloured	100	7

The black samples (although only two in number - samples F6 and F26) have excessively high mercury contents (>5000µg/g), and a correspondingly high sulphur content (ca.2000µg/g). It is likely that these samples are deposits of mercury sulphide (metacinnabar), presumably following exposure of the sample to a polluted urban atmosphere (the reaction of mercury (II) chloride with sulphur from the burning of fossil fuels). It is also possible that the sulphur has been directly applied to the samples at some stage through treatment with carbon disulphide. This was a common practice in foreign herbaria (both F6 and F26 originate from Canada). Sample F18, (see Table 8 note), a Hungarian specimen, had information accompanying the specimen that stated it had been treated with both carbon disulphide and phosphine.

The orange samples have shown some indication of representing higher mercury content than the other colour groups, with the majority of the samples (88%) having a mercury content >200µg/g, but the sample number is too low to be held as truly representative of the group (n=8). Similarly, the majority of peach samples (79%) have a mercury content >200µg/g. It is possible that the orange and peach samples containing little or no mercury have been misclassified, as foxing also fluoresces peach or orange under UV-A light. Foxing, however, is also apparent under visible light, and hence differs from the fluorescence being studied in this research. Nevertheless, if the visible signs of foxing were missed, misclassification could have resulted.

The white samples have shown little difference in their mercury distribution to the non-coloured areas, and the occurrence of the colour seems unlikely to be due to the presence of mercury. Only 26% of this sample group has a mercury content higher than observed in the non-coloured areas (Table 31).

Of the three non-fluorescent coloured areas, high mercury content is certainly a common factor, but it is difficult to be definitive with the large proportion of samples in the grey and brown groups showing little or no mercury content at all. Although 32% of the samples in the grey group has relatively high mercury content (200–3000µg/g), 55% has less than 20µg/g, and cannot be distinguished from the non-coloured areas. Similarly, 42% of the brown group has relatively high mercury content (150–4000µg/g), with 42% having less than 20µg/g. Both grey and brown areas were apparent under visible light appearing as marks on the paper. Like foxing, these marks may not be significant in terms of historic residues, but may coincide with the presence of biocide residues.

The arsenic and lead distributions for the colour groups have shown no evidence that the observed colours are associated with the presence of these two metals (Figs 49 and 50, respectively).
It is clear from the elemental frequency distributions that the development of four of the coloured fluorescent areas (cream, peach, yellow and orange) on the specimen sheets is associated with the presence of mercury. There is little to suggest, however, that there is any significant difference between these four colours in terms of their mercury concentrations. This may be associated with the extremely subjective nature of the colour classification process. The colours observed under UV-A light were graded against the Munsell colour chart (Munsell, 1969), viewed alongside in visible light. This evaluation was highly subjective, and may have led to discrepancies in colour assignment. It must be considered more likely, however, that differing mercury concentrations is not the key distinction between these four colours, particularly since the accelerated ageing tests revealed a time-dependent colour change from cream to peach, with no apparent change in mercury concentration.

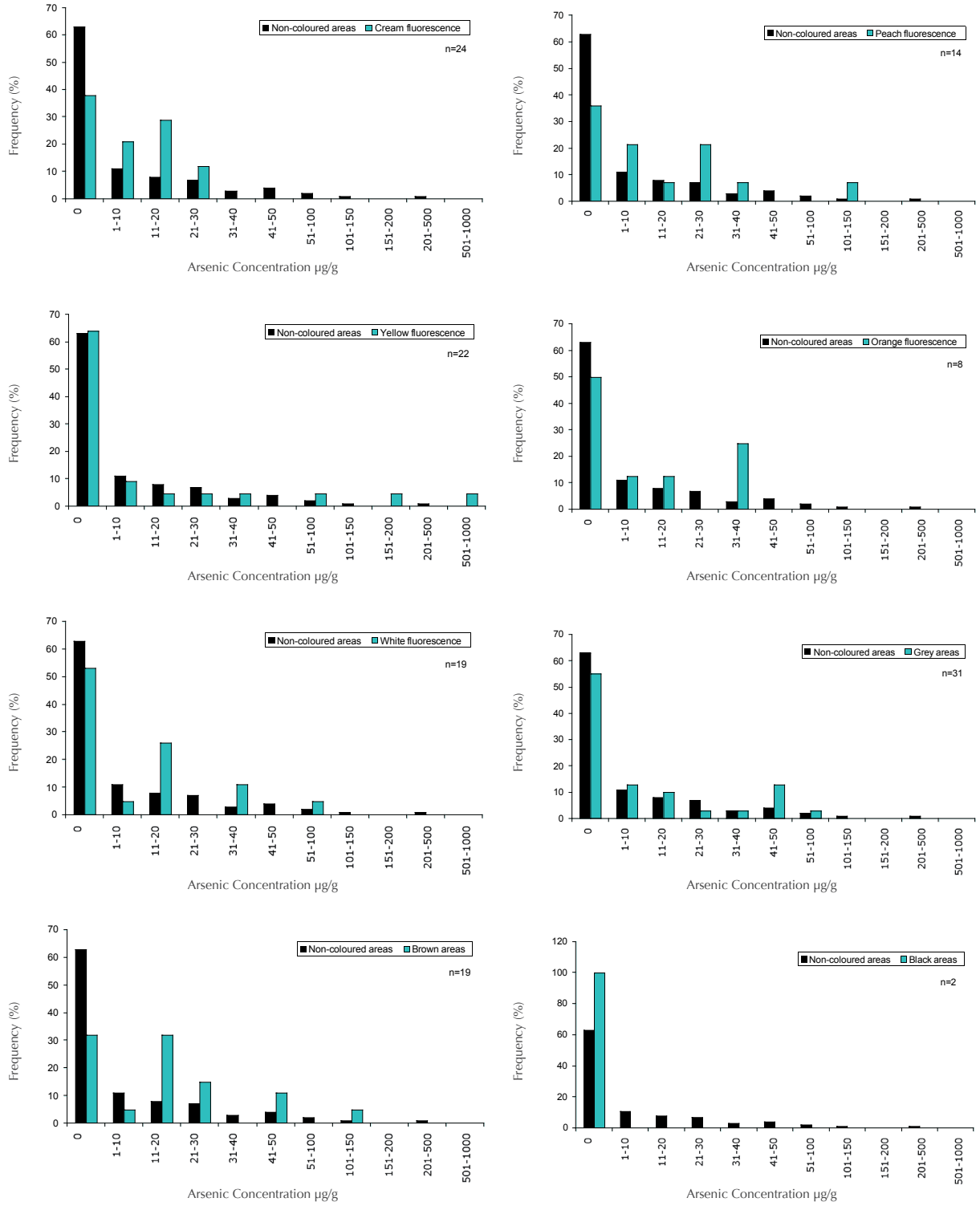

Figure 49 Frequency distributions of arsenic content within the eight different coloured areas identified under UV-A light. Comparison is made with the frequency distribution observed for 119 non-coloured areas.

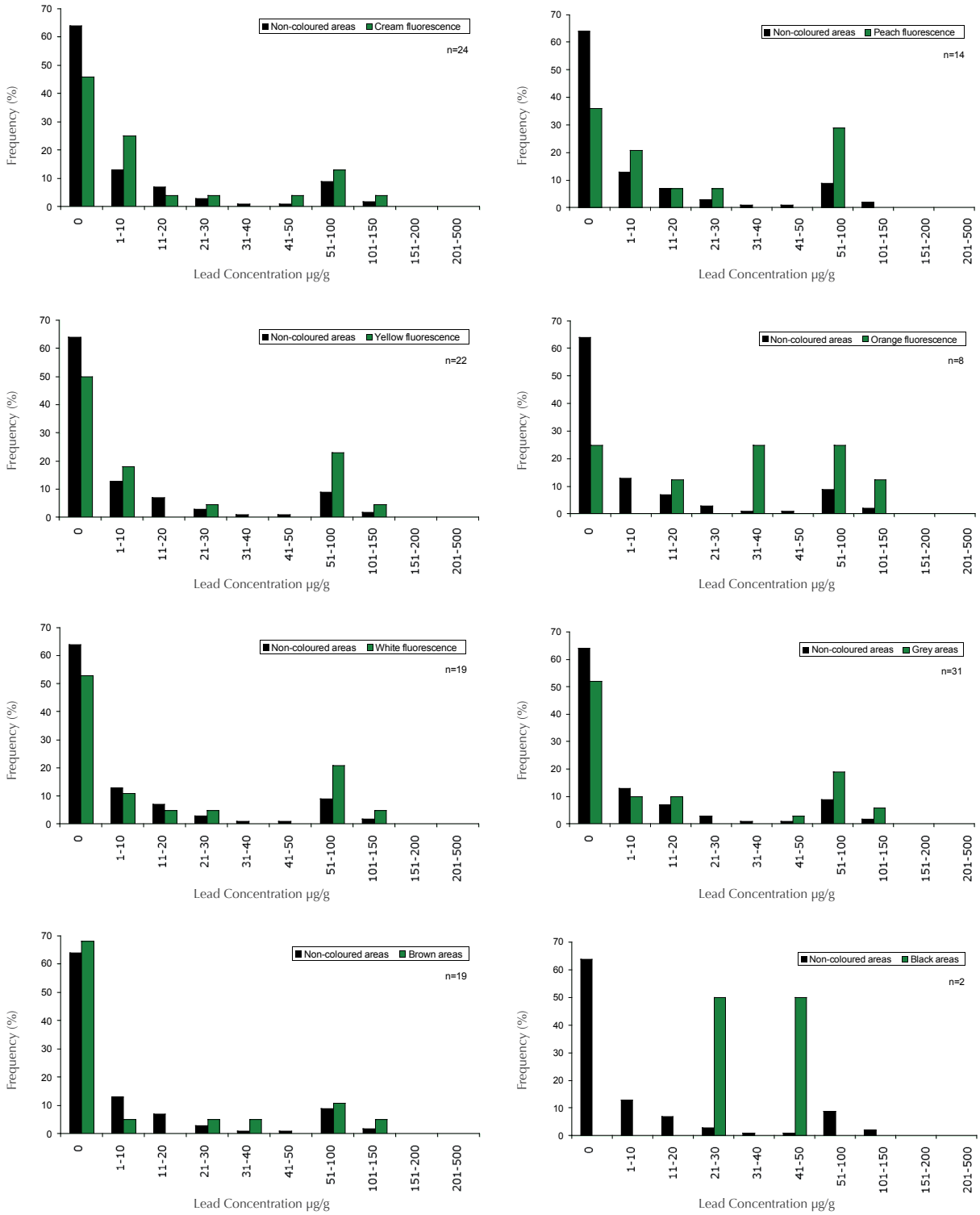

Figure 50 Frequency distributions of lead content within the eight different coloured areas identified under UV-A light. Comparison is made with the frequency distribution observed for 119 non-coloured areas.

Although the inhomogeneity within the colour groups has meant that multivariate statistical methods were inappropriate for identifying discriminating factors between the groups, cluster analysis (CLUSTAN, 2001) was employed on the colour dataset to ascertain whether any relationships, other than colour, could be identified between the samples analysed.

Cluster analysis was carried out on the coloured dataset from the PIXE analysis (initially n=139; v=7: Hg, As, Pb, Ba, S, Cl, Si). Appropriate data transformation for trace elemental concentrations (z-scoring) was carried out before analysis. The results indicated that three discriminating elements were present within the dataset: mercury, sulphur and barium.

Barium was identified as a discriminating element on the basis that it is only present in a small set of samples (16 out of 55 specimen sheets) (see Table 32).

Table 32 Comparison of samples containing barium

Specimen sheet	No of samples	Date	Previous herbarium	Country	Collector	Paper density (g/m^2)
2	2	1894	Shoolbred WA	UK	Evans HA	205
7	4	1933	Oxford University	UK	E.G.A.	192
8	3	1923	Oxford University	UK	Ex Queen Mary College	195
17	2	1933	Oxford University	UK	Inter Arts, Science and Agriculture	147
19	3	1930	Oxford University	UK	Ex Queen Mary College	198
52	17	1952	Edees, ES	UK	Daltry, HW	138
59	2	1898	Steinberg, O.	Switzerland	Steinberg	162
76	4	1931	Petropolitano	Russia	A. Henry	204
F06	1	1882	Museum of Nature, Ottawa	Canada	Macoun, J.	129
F10	1	1875	Museum of Nature, Ottawa	Canada	Macoun, J.	74
F14	4	1884	Museum of Nature, Ottawa	Canada	Bell, R.	141
F18	2	1840	Hungarian Botanical Institute	Budapest	Muller, B.	112
F20	4	1896	Belarus University	Belarus	Callier, A	147
F21	2	1950	Belarus University	Belarus	–	153
F22	2	1949	Belarus University	Belarus	–	173
F26	2	1883	National Museum of Canada	Canada	Macoun, J.	164

The only cited barium-containing biocide is barium fluorosilicate (Hall, 1988, Bridson and Forman, 1998). Although this was used as a pesticide, it was not applied directly to the herbarium specimen – it was applied, in the form of a sugared paste, to the underside of shelves and the inside of wooden cabinets.

Baryte ($BaS0_4$) was introduced into the paper-making process during the 1820s and would, therefore, be expected to be present in all of the samples, with the exception of the 18th century Bute samples, glue and the specimen. Its occurrence is widespread in good quality papers nowadays (Blanc Fine™), although it is a costly addition (Beazley, 1991).

Although barium was not detected across the whole sample set by PIXE, the AAS analyses revealed that the barium occurrence was more widespread. The AAS analysis, however, is a bulk analysis, with the samples being pre-digested, and is dominated by paper and its constituents. PIXE, on the other hand, is essentially a surface technique, and will predominantly determine the composition of the surface layers and any treatments applied.
In the samples that it occurs, barium is present in all areas (coloured and non-coloured) and is associated with the specimen sheet as a whole. There is no association, therefore, between the presence of barium and the observed coloured areas on the specimen sheets.
The barium-containing samples identified by PIXE (Table 32) have shown a vast spread in barium content (50-35,000µg/g). Although a number of the samples are from foreign collections, there is no obvious link between them to explain why this group of samples is different.

As discussed previously, of the 139 coloured samples analysed, 59 were undistinguishable from the non-coloured samples (Table 31). These samples were removed from the dataset. The two main discriminating elements identified for the remaining 93 coloured samples were mercury and sulphur. After the removal of outliers, cluster analysis identified 12 main groups (Table 33), with clusters 1–8 being significantly different to clusters 9–12 (see Figure 51).

Comparison of the cluster members has shown no link between age, paper type, or specimen, but there is a strong indication that the Canadian-based collections are significantly different to the UK-based collections – 83 % of the Canadian samples have been separated by the cluster analysis – six of the 12 clusters are Canadian based (Clusters 5 and 8–12). Furthermore, within these clusters, there is some indication that the grey and black coloured areas are distinctive. It should be noted, however, that the number of specimen sheets represented is small, and these results cannot be extrapolated with any confidence beyond the samples analysed (Figure 51).

The discriminator between the Canadian and UK samples is sulphur, with the exception of Cluster 8, which can be discriminated by both sulphur and mercury. In general, the Canadian samples are defined by significantly higher sulphur content than the UK samples (although the data is still very spread, and the Canadian clusters are only loosely defined). As discussed previously, the high sulphur content may be due to sulphur having been directly applied to the samples, at some stage, through treatment with carbon disulphide. This was a common practice in foreign herbaria.

Table 33 Membership of the 12 clusters identified by cluster analysis of the PIXE dataset.

Cluster	Cluster Membership					
	Sample No	**Colour**	**Date**	**Lignin**	**Type status**	**Collection Origin**
1						
	Ad	Cream	1932	✓	–	UK
	19f	Yellow	1930	–	–	UK
	48d	Yellow	1893	✓	Isotype	UK
2	F11e	Brown	1878	–	–	CANADA
	08e	Cream	1923	–	–	UK
	21c	Orange	1895	–	Lectotype	UK
	76f	Orange	1931	✓	–	RUSSIA
3	03c	Brown	1878	–	–	UK
	28a	Brown	1895	–	–	UK
	F09d	Brown	1905	–	–	CANADA
	06d	Cream	1938	–	–	UK
	07d	Cream	1933	–	–	UK
	76e	Cream	1931	✓	–	RUSSIA
	06e	Grey	1938	–	–	UK
	F14f	Orange	1884	–	–	CANADA
	02e	Peach	1894	–	–	UK
	18e	Peach	1861	✓	–	UK
	21d	Peach	1895	–	Lectotype	UK
	52k	Peach	1952	✓	–	UK
	76g	Peach	1931	✓	–	RUSSIA
	03i	White	1878	–	–	UK
	11e	Yellow	1935	–	–	UK
	52l	Yellow	1952	✓	–	UK
4	19j	Orange	1930	–	–	UK
	48c	Peach	1893	✓	Isotype	UK
	08f	Peach	1923	–	–	UK
	Bu c06	White	1760s	–	–	UK
	76h	Yellow	1931	✓	–	RUSSIA

Table 33 (continued)

Cluster	Cluster Membership					
	Sample No	**Colour**	**Date**	**Lignin**	**Type status**	**Collection Origin**
5						
	F26b	Cream	1883	_	_	CANADA
	F06e	Grey	1882	_	_	CANADA
	F26d	Grey	1883	_	_	CANADA
	F26e	Grey	1883	_	_	CANADA
	F06f	Peach	1882	_	_	CANADA
	01g	White	1872–1918	_	_	UK
	F06g	Yellow	1882	_	_	CANADA
6	F07w	Grey	1800	_	_	CANADA
	52j	Orange	1952	✓	_	UK
	11d	Peach	1935	_	_	UK
	Bu c05	White	1760s	_	_	UK
	52m	Yellow	1952	✓	_	UK
	52n	Yellow	1952	✓	_	UK
	52o	Yellow	1952	✓	_	UK
	52p	Yellow	1952	✓	_	UK
7	F11g	Cream	1878	_	_	CANADA
	Bu c07	Grey	1760s	_	_	UK
	11c	Orange	1935	_	_	UK
	F11h	Yellow	1878	_	_	CANADA
	52q	Yellow	1952	✓	_	UK
	52r	Yellow	1952	✓	_	UK
	52s	Yellow	1952	✓	_	UK
8	F06c	Black	1882	_	_	CANADA
	F26a	Black	1883	_	_	CANADA
9	F07e	Brown	1800	_	_	CANADA
	F07f	Brown	1800	_	_	CANADA
	F07g	Brown	1800	_	_	CANADA
	F07h	Brown	1800	_	_	CANADA
	F07t	Cream	1800	_	_	CANADA
	F07u	Cream	1800	_	_	CANADA
	F07v	Cream	1800	_	_	CANADA
	F13q	White	1877	_	_	CANADA
10	F07h	Brown	1800	_	_	CANADA
	F07i	Brown	1800	_	_	CANADA
	F07j	Brown	1800	_	_	CANADA
	F07q	Cream	1800	_	_	CANADA
	F07r	Cream	1800	_	_	CANADA
	F07s	Cream	1800	_	_	CANADA
11	F13j	Grey	1877	_	_	CANADA
	F13k	Grey	1877	_	_	CANADA
	F13l	Grey	1877	_	_	CANADA
	F13m	Grey	1877	_	_	CANADA
12	F13n	Grey	1877	-	_	CANADA
	F13o	Grey	1877	-	_	CANADA
	F13p	Grey	1877	-	_	CANADA

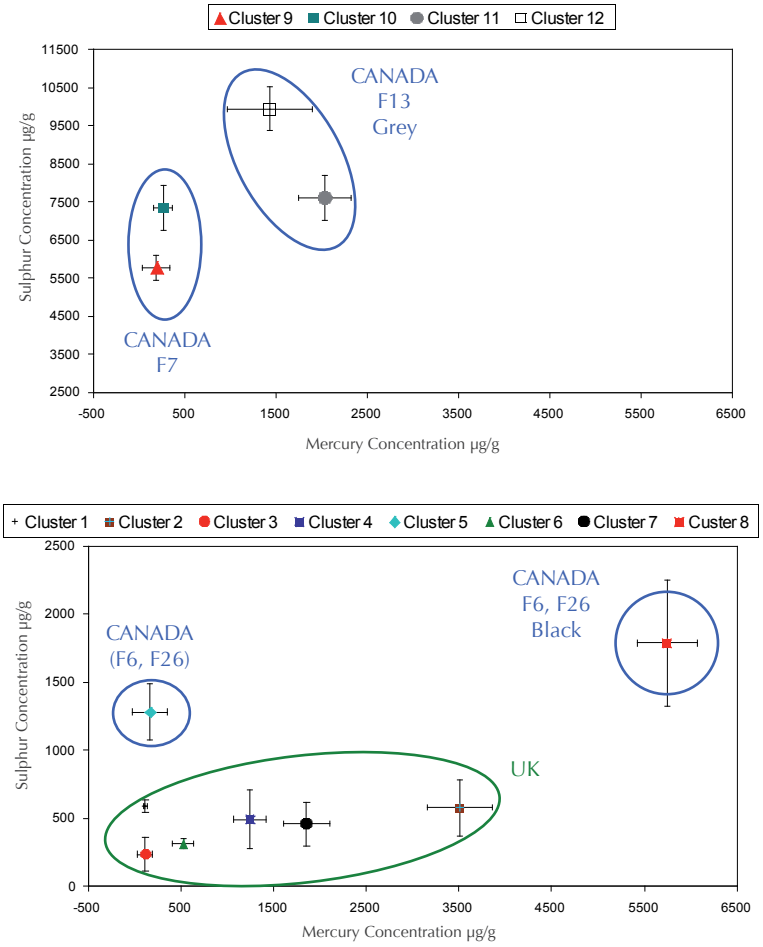

Figure 51 Bielemental plot of the two main discriminating elements (mercury and sulphur) identified from cluster analysis of the PIXE dataset. The twelve main clusters identified are given. The country of origin of the collection is also indicated.

There is no evidence within the UK samples to suggest that the differences between the 6 clusters are real. The apparent, loose, correlation between mercury and sulphur concentrations can be reasonably attributed to non-uniformity in biocide application. Correlated data, however, cannot be adequately analysed by cluster analysis, as the tendency is to divide the population into clusters along the correlation line. In the absence of any identifiable relationships between the cluster members, this is likely what is being observed in the UK data, given in Figure 51.

7.3 Fluorescence Development

It has been clearly demonstrated that the development of the fluorescent areas on the specimen sheets is due to the presence of mercury, but there is little evidence to suggest that the variation in observed colour is linked to the concentration. On the contrary, the accelerated ageing tests indicated that the colour is more likely to be an artefact of time, and hence may be a result of a chemical reaction during paper degradation.

The chemistry of cellulose degradation is fairly complex, but one of the main reactions occurring in the ageing specimen sheets is oxidative degradation. It is generally accepted that autoxidation of cellulose occurs with atmospheric molecular oxygen under ambient conditions via a free-radical chain mechanism (Figure 52). The rate of reaction is very slow due to the low redox potential of O_2 (reaction scheme 1), but is promoted by alkaline conditions (reaction scheme 2) and also by ultra-violet radiation.

Initiation	$RH + O_2$	→	$R^{\bullet} + HOO^{\bullet}$	1
	$R^- + O_2$	→	$R^{\bullet} + O_2^{-\bullet}$	2
Propagation	$R^{\bullet} + O_2$	→	$ROO^{\bullet}$	3
	$ROO^{\bullet} + R'H$	→	$ROOH + R'^{\bullet}$	4
	$ROO^{\bullet}$	→	$R^+ + O_2^{-\bullet}$	5
	$R^{\bullet} + R'H$	→	$RH + R'^{\bullet}$	6
	$RH + HO^{\bullet}$	→	$R^{\bullet} + HOH$	7
Termination	$R^{\bullet} + HO^{\bullet}$	→	ROH	8
	$R^{\bullet} + O_2^{-\bullet}$	→	ROO^-	9
	$R^{\bullet} + R^{\bullet}$	→	R–R	10

Figure 52 Possible free-radical chain mechanism of the oxidation of cellulose

Due to the variety of redox potentials of active oxygen species, a large number of reactions with cellulose and lignin are possible. Molecular oxygen itself may be reduced to water via a 4-electron stepped reaction sequence, giving rise to several intermediate products: superoxide (O_2^- / HOO); hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radicals ($HO^{\bullet}$), which can further propagate the oxidative processes (reaction scheme 7).

The inevitable presence of transition metals in the paper can offer a number of additional reactions (Figure 53). The metal-catalysed homolytic decomposition of peroxides – the Fenton reaction (reaction scheme 11) and the oxidative decomposition of peroxides (reaction scheme 12) both produce additional free radicals to further propagate the oxidation of cellulose.

$HOOH + M^{n+}$	→	$M^{(n+1)+} + HO^{\cdot} + OH^{-}$	11
$HOOH + M^{(n+1)+}$	→	$M^{n+} + HOO^{\cdot} + H^{+}$	12
$HOO^{\cdot} + M^{(n+1)+}$	→	$M^{n+} + O_2 + H^{+}$	13

Figure 53 Transition metal reactions likely to occur in paper during oxidative degradation of cellulose

Reaction schemes 12 and 13 (Figure 53) indicate that transition metals are likely to be reduced by the peroxide and superoxide species produced during the oxidative processes associated with cellulose degradation. Both iron(III) and copper(II) are known to undergo these reductive reactions. It is, therefore, likely that in biocide applications, the mercury(II) (in mercuric chloride) will be reduced to Hg(I) in accordance with reaction schemes 12 and 13.

Mercury(I) chloride (Hg_2Cl_2) is the mineral calomel, closely associated with the other mercury-containing minerals, such as metacinnabar (HgS). Unlike mercuric chloride (which is always white), calomel ranges in colour from white to yellow to grey, and is often a fluorescent red under UV light. It is possible, therefore, that variation in the concentration of Hg(I), and hence in the extent of reduction of Hg(II) to Hg(I), across a specimen sheet, may well give rise to a range of colours, mostly in the red spectral region (cream, yellow, peach, orange) and mainly fluorescent in nature. This may also explain the white and grey coloured areas observed.

The fluorescent areas that developed during the accelerated ageing tests were submitted to X-ray photoelectron spectroscopy (XPS) to determine the mercury speciation, and hence establish whether the Hg(II) biocide application was being reduced during the ageing process. In all three samples tested (Table 34), Hg(I) (and also Hg(0) in sample AA1a) was detected.

Table 34 Observed fluorescence and mercury valence state (XPS) on samples after accelerated ageing at 80°C with no RH control

Sample	Chemical Application	Aged	Observed Fluorescence	Hg valence state
AA1a	Mercuric chloride	3 days	Cream	0 / +1
AA1b	Mercuric chloride	3 days	Cream	+1
AA2a	Naphthalene	3 days	None	N/a
AA3a	Mercuric chloride and Naphthalene	3 days	Peach	+1
AA4a	None	3 days	None	N/a

Of the six coloured areas analysed (five fluorescent and one grey), three were found to contain the Hg(I) species (Table 35); two contained Hg(II) and the grey sample had no mercury present. The non-coloured areas gave no indication of the presence of mercury at all. The samples from herbarium sheet 19 (dating from 1930) were indeed fluorescing, but the XPS results indicated that the mercury was present as Hg(II) in all cases. This is difficult to explain. It is possible that the cellulose degradation of this sheet has not progressed far enough to have caused complete reduction of the Hg(II), leaving a significant Hg(II) content to dominate the XPS analysis, but sufficient Hg(I) to cause the observed fluorescence. It is also possible that due to the inhomogeneity of the applied treatment, and the small sample area analysed by XPS, the fluorescing area may have been missed by the analysis. Misassignment of the mercury peak is also a possibility: interference from silicon causes broadening of the Hg(4f) peak. Since the binding energies of Hg(I) and (II) are very close (100.8 and 101.4 eV, respectively), successful differentiation requires good resolution (i.e. narrow peaks). Herbarium sheets A (1932) and F14 (1884) contained the Hg(I) species.

Table 35 Mercury speciation present within fluorescent areas on a range of herbarium sheets. Mercury standards are also given.

Sample	Fluorescence	Binding Energy (eV)	Valence State	Date
19h	Non-coloured	Not found	–	1930
Ae	Non-coloured	Not found	–	1932
F14h	Non-coloured	Not found	–	1884
19i	Yellow	101, 101.5	+2 +2	1930
19j	Orange	101.2, 102.1 101.6	+2 +2	1930
Af	Yellow	100.3	+1	1932
Ag	Peach	100.9	+1	1932
F14i	Brown	100.7	+1	1884
Hg (II)	n/a	101.4	+2	
Hg (I)	n/a	100.8	+1	
Hg (0)	n/a	99.5	0	

The XPS analysis provided further information about the pesticide applications made to the collections. Nitrogen, chlorine and sulphur were identified in addition to the presence of mercury. The presence of chlorine and sulphur would be expected from the mercury (II) chloride treatment and atmospheric sulphur, although sulphur could also have been incorporated through the paper- making process, or through additional treatments (e.g. CS_2). The nitrogen, however, was present in the form of an amide/amine functional group, with high concentrations observed in samples Af, F14h,i and 19h,i and j. None was observed in the artificially-aged samples. It is possible that mercuric nitrate ($Hg(NO_3)_2$) was

used as a pesticide at some point within the collection, as it is known to have been used in natural history collections, particularly abroad.

From the research, there is compelling evidence to support the hypothesis that the observed fluorescence within the herbarium collection is due to the reduction of Hg(II) to Hg(I) during the oxidative degradation of cellulose, occurring as part of the natural ageing process. Thus, the observation of fluorescence within herbaria ought to be indicative of mercury contamination. Further supporting evidence for this hypothesis is obtained from the observed increase in the rate of fluorescence development in the presence of naphthalene.

The oxidative decomposition of naphthalene is observed in many systems that produce hydroxyl radicals (Hykrdova *et al*, 2002). Although this reaction is usually initiated by the homolytic decomposition of hydrogen peroxide by UV irradiation, hydroxyl radicals are readily produced during the autoxidation of cellulose, as an intermediate in the reduction of molecular oxygen. For herbarium sheets that have been exposed to naphthalene, the autoxidation of the cellulose will readily initiate the oxidative decomposition of the naphthalene. It is believed that the mechanism of naphthalene decomposition involves the production of over 50 intermediates, including the formation of many peroxyl radicals. The mechanism is believed to proceed via a number of steps that involve the elimination of hydroperoxyl radicals (HOO$^{\prime}$), which further increases the probability of the reduction of Hg(II) via reaction scheme 13 (Fig. 53), and explains the more rapid development of the peach fluorescence in the presence of naphthalene during artificial ageing.

7.4 A Rapid Screening Method for Detection of Mercury-contaminated Collections

From the results obtained to date, it is evident that a hand-held UV-A lamp can provide a rapid and effective method of identifying samples within a collection that are contaminated with mercury, thus providing a rapid and economical means to prioritise which collections require immediate re-mounting. Furthermore, this method will enforce the implementation of safe, standard procedures to protect personnel and visitors when handling the collections, plus enable the removal of a large amount of hazardous chemical from the herbarium environment.
Some key institutions, such as the RBG, Kew, Natural History Museum and Linnean Society, do not support re-mounting. They regard the mount sheet to be as important as the specimen itself, providing information on the quality of paper, and its age, through watermarks, chain and laid lines and fibre content. Stains and marks are regarded as historic, so even biocide applications are regarded as archival and would not be removed. Conservation is frequently thwarted with such ethical constraints.

The NMW, however, does not re-mount indiscriminately. Removing the original backing sheet will cause the loss of part of the specimen's original history and is therefore carefully considered. The third Earl of Bute herbarium, for example, dates back to the mid 1700s and was owned by a highly prominent politician (the Prime Minister, 1762) and botanist, who was strongly linked to the city of Cardiff. Bute's herbarium papers can be viewed as being of significant value, and to destroy the mount

sheet would cause the loss of information, including the type and quality of the paper, the watermark which provides information regarding the identity of the paper mill, how it was made and the type of fibres used. Additionally, the method of mounting is of historic value, as the collection has been mounted with sealant wax and paper strips; no other collection within NMW has been mounted in this way. The collection shows some grey staining, and in some cases, the biocide stains have become a part of the archival history of the collection (Ashdown and Gosnay, 2009). It is therefore a very difficult decision that has to be made. If the specimen and mount sheet are kept together then there is always going to be health issues associated with handling and working with these collections.

The NMW view is to assess the risks to health. With regard to the older collections, these will have had a significant application of biocide and it is probable that lead, arsenic and mercury will be present. With these specimens, however, the sheets frequently illustrate grey staining, suggesting a heavy metal sulphide which is less volatile due to the strong associations these metals have with sulphur.

The majority of the NMW herbarium is dated c.1880-1970s and the papers were generally more modern, and hence less stable, and far less historic information can be gleaned from them. All data associated with the specimen is carefully removed and placed with the specimen on its new mount sheet, so no written data is ever lost.

7.5 Laser Ablation Decontamination

Prior to this research, there was little information regarding the amount of mercury that could be released from contaminated sheets. Measurement of the air quality within the herbarium was often not conducted during normal conditions, and therefore accurate assessment of the hazard was not possible. This research has helped to provide data to explain the behaviour of the most toxic elements within the collection, and allow the assessment of risk to staff and visitors handling the collection.

Measurement of mercury vapour emitted from the sheets shows that mercury concentrations of c.1000ppm on the specimen sheets, results in c.0.7ppm (5.74mg/m^3) mercury vapour in the immediate environment. This is nearly 300 times higher than the recommended TWA for mercury and mercuric chloride (0.02mg/m^3).

With an average mercury concentration of 450ppm within the orange fluorescence, it is assumed that a minimum of 2.5 mg/m^3 is being released from these sheets. Although an estimation, this data provides the means to assess the likely exposure to mercury a handler would receive from these sheets, and steps can be taken, accordingly, to reduce this risk.

Disposal of contaminated sheets, following re-mounting, is known to be costly. If materials contain >0.1% (1000ppm) of arsenic, lead or mercury, the material has to be taken to a contaminated landfill site. Transportation is also costly, as only licensed contractors can carry contaminated waste. This

research has helped to determine how the sheets have been contaminated and to what extent.

For those collections that cannot be re-mounted, laser ablation decontamination may be a possible alternative.

Elemental analysis of the Bute sample (Bute Hb, c.1770) indicated that significant concentrations of lead, arsenic, sulphur and chlorine were present prior to the laser ablation treatment. Mercury and barium were not detected.

The laser ablation successfully reduced the lead content of the herbarium sheet by 62%. This reduction was substantial, and laser ablation should be considered a valuable method of decontamination for lead in paper. Laser ablation had no affect on the arsenic concentration, but the lighter elements were much more effectively removed – the sulphur and chlorine content were greatly reduced (48% and 20%, respectively), and some of the discolouration, therefore, removed. Aesthetically, the visual difference after cleaning was noticeable, but not all of the dark grey stain, apparent on the herbarium sheet, was removed.

Dark grey deposits on herbarium sheets were previously only associated with mercury (metacinnabar) (Hawks and Bell, 1999, Sirois and Helwig, 1999). As mercury was not detected on the Bute sample, the grey discolouration, in this case, may be due to another heavy metal sulphide.

Analysis (SEM-DX) of a specimen, *Equisetum* sp., dated around the 1660s, with characteristic dark spots on the paper around the specimen, and shiny, grey metallic regions, revealed the presence of lead (Fig. 54).

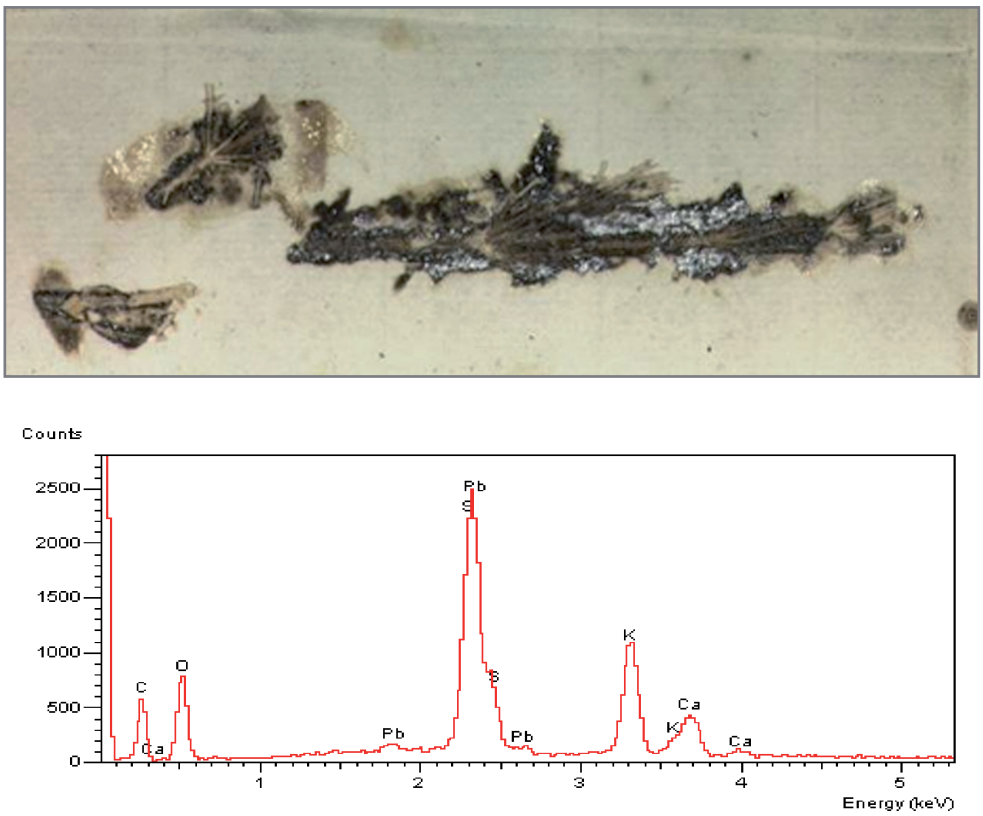

Figure 54 Specimen *Equisetum sp.* circa 1660, illustrating dark spots on paper, and grey metallic areas (top). The X-ray spectrum (below) clearly indicates the presence of lead.

The presence of lead in this specimen indicates that other heavy metals may produce the characteristic dark grey staining often associated with metacinnabar and that it is not necessarily an indication of mercury contamination.

This study has been extremely informative and has indicated that, for valuable material, laser ablation may be a resourceful method to reduce lead contamination and the staining present on certain areas, or over data labels. Historic sheets, such as the third Earl of Bute herbarium, would benefit from this microdestructive decontamination method, as the specimen sheet, original data and the method of mounting are all important to the original integrity and history of the specimen. In such cases as this, laser ablation can provide a method of reducing the dark, unsightly marks on the sheets that may obscure data or hand writing.

8 Conclusions

A number of key conclusions can be drawn from the research:

- The NMW herbarium is contaminated with mercury, arsenic and lead.

- Although the metals are distributed inhomogeneously across the specimen sheets, it has been demonstrated that the development of the coloured / fluorescent areas is directly linked to the presence of mercury. The presence of arsenic and lead plays no part in the development of coloured / fluorescent areas.

- There is compelling evidence to support the hypothesis that the observed fluorescence within the herbarium collection is due to the reduction of Hg(II) to Hg(I) during the oxidative degradation of cellulose, occurring as part of the natural ageing process. Thus, the observation of fluorescence within herbaria ought to be indicative of mercury contamination.

- Both accelerated ageing tests, and empirical observations, indicate that the coloured / fluorescent areas take at least 30 years to develop, as the degradation of cellulose has to progress sufficiently to propagate the production of the fluorescent Hg(I) species.

- The application of naphthalene as a biocide is very common, and is likely to be present in the majority of herbaria in Britain and abroad. The presence of naphthalene increases the rate of fluorescence development on specimen sheets that have also been treated with mercuric chloride. The oxidative decomposition of naphthalene is a source of additional hydroperoxyl radicals, also produced during the oxidative degradation of cellulose. These hydroperoxyl radicals are responsible for the reduction of Hg(II).

- It seems likely that a hand-held UV-A lamp provides a rapid and effective method of identifying those samples within the collection that have been highly contaminated with mercuric chloride, and provide a means to prioritise which collections require immediate re-mounting. Furthermore, this may inform the implementation of standard procedures to protect personnel and visitors handling the collections, and enable the removal of a large amount of hazardous chemical from the herbarium environment.

PART III: ORGANIC RESIDUES – NAPHTHALENE

9 Experimental

9.1 Sample Selection

Thirty-one herbarium sheets were taken from cupboard 57 of the NMW herbarium (Table 36). All cabinets within the NMW herbarium have been treated with naphthalene pellets in the past, the naphthalene having been placed in nylon bags and attached to the inside of the cabinet doors. Although the amount of naphthalene in each bag was not measured precisely, and hence the concentration within each cabinet was expected to vary, all of the herbarium material had been subjected to naphthalene contamination. Cabinet 57 was chosen for this research since the air within had been analysed previously during air monitoring. The air-borne naphthalene concentration in Cabinet 57 was measured as 0.62mg/m^3 (Table 5).

Table 36 Summary of the herbarium mount sheets selected for the naphthalene studies

Sample	Accession no	g/m²	Lignin	Date	Comments	Method
A1	86.81.1428	149	X	1977	Thick, finished	Air-dried
A2	86.81.1459	135	X	1937	Thick, finished	Air-dried
A3	Outer sleeve of 1459	85	X	1937	Thin, coarse	Air-dried
A4	26.102.16	225	X	1926	Finished	Method development
D1	86.81.3497	130	X	1956	Thin, finished	Distribution
D2	27.72.1383 [1]	107	X	1891	Fine paper	Distribution
D3	27.72.1383 [2]	90	X	1891	Fine paper	Distribution
D4	27.72.1383 [2]	90	X	1891	Fine paper	Distribution
F1	20.7	121	X	1864	Unfinished paper	Freeze-dried
F2	25.149.3017	118	X	1969	Finished, cotton fibre, non gelatine size, rosin	Freeze-dried
F3	86.81.1456	165	X	1961	Thick, unfinished	Freeze-dried
F4	86.81.1450	131	X	1968	Thick, finished blk tape	Freeze-dried
F5	42.138.467	206	X	1928	Glossy finish	Method development
F6	25.562.61	100	✓	1925	Finished	Method development
H1	86.81.1442	128	X	1965	Heavy, finished	Oven-dried
H2	86.81.1458 C	90	X	1975	Thin, unfinished	Oven-dried
H3	86.81.1458W	155	X	1975	Thick, finished, blk plastic	Oven-dried
H4	86.81.1671	138	X	1929	Thick, finished	Oven-dried
H5	64.74.18	200	X	1963	Heavy card	Method development
H6	35.435.3	105	X	1935	Finished	Method development
H7	28.131.5200	103	X	1891	Finished	Method development
H8	28.131.2905	103	X	1891	Finished	Method development
H9	25.530.579	133	✓	1925	Finished	Method development
H10	23.94.1708	132	✓	1909	Unfinished brittle.	Method development
H11	25.149.5494	103	X	1892	Fine paper	Method development
H12	27.72.1383 1	107	X	1891	Fine paper	Method development
M1	35.435.1	115	✓	1930	Unfinished	Method development
M2	60.341.21	105	✓	1958	Unfinished	Method development
M3	29.149.5494	57	X	1892	Fine paper	Method development
M4	87.b	146	✓	1885	Thick, finished	Method development
M5	25.562.21	134	✓	1921	Thick, finished	Method development

9.2 Method Development

In order to evaluate a number of decontamination methods, it was essential to develop a method for the accurate and precise analysis of naphthalene on the herbarium sheets.

A standard analytical technique for naphthalene is gas chromatography with flame ionisation detection (GC-FID). Bianchi *et al* (2005) achieved limits of detection for naphthalene of 0.43 ppm and limits of quantification of 0.72 ppm ± 0.5%. GC-FID was therefore deemed to be an appropriate technique, and thought to be sufficiently sensitive for this research.

The instrument used was a Varian 3800 with a Perkin Elmer Gas chromatography flame ionisation detector. A DB 1ms column was used, comprising 100% dimethylpolysiloxane, which is non-polar with low bleed characteristics. Calibration was achieved by preparing a series of naphthalene solutions (10-50 ppm) in CS_2. An aliquot (1 ml) of each solution was taken and mixed with an equal volume of *o*-xylene (20 ppm in CS_2) as the internal standard (Figures 55 and 56). Calibration was effected prior to each sample run.

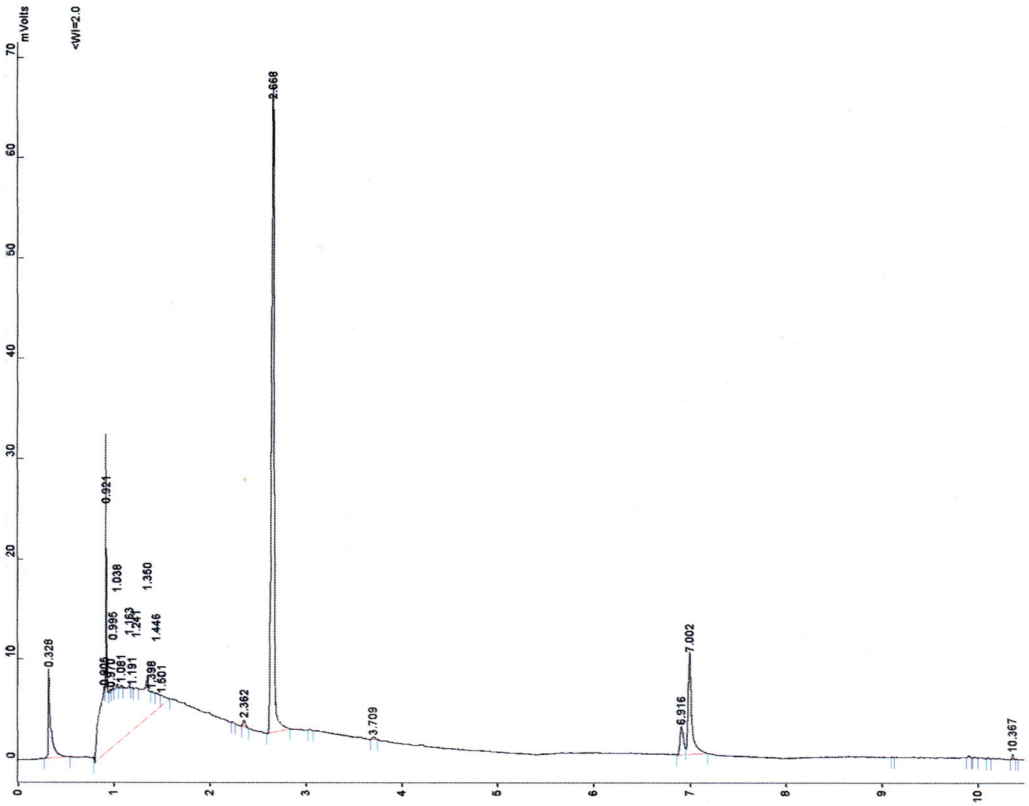

Figure 55 Annotated chromatogram showing the resolution of the *o*-xylene (RT 2.668) and naphthalene peaks (RT 6.916 and 7.002) for 250ppm naphthalene standard with 1µl *o*-xylene. X axis shows Time (mins) against y Response (mV).

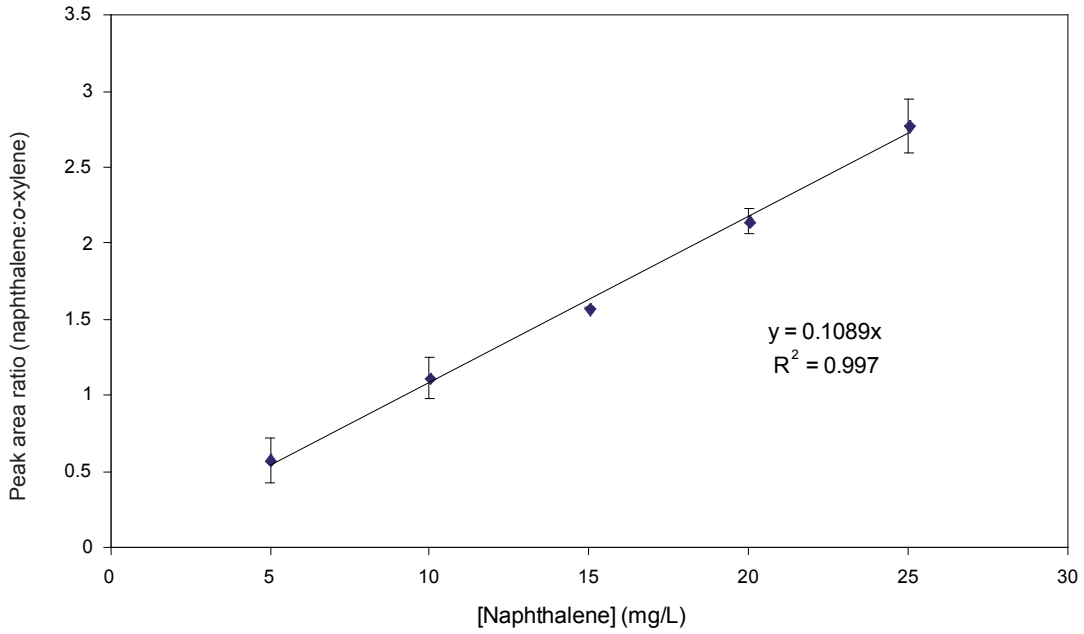

Figure 56 Typical calibration curve for naphthalene (n=3) by GC-FID, using *o*-xylene as internal standard.

The limit of detection for naphthalene by the GC-FID can be determined by calculating the mean background measurement in the region of the peak of interest. The limit of detection is defined as:- *the mean background + 3 standard deviations*.

In order to determine the limit of detection for the method, five random chromatograms were taken, and the background areas determined on either side of the main naphthalene peak (Fig. 57, Table 37).

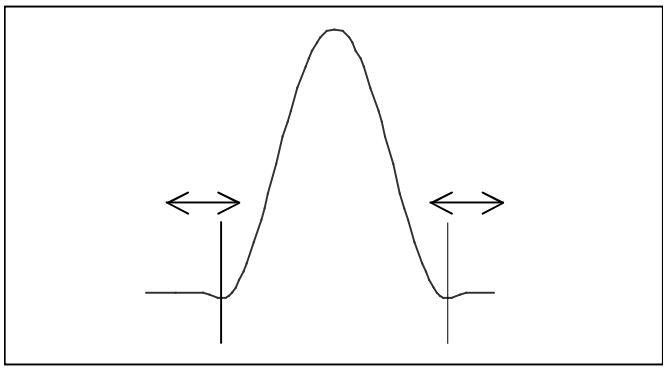

Figure 57 Diagram showing main naphthalene peak and peak areas either side of measured area

Table 37 Background areas determined from either side of the main naphthalene peak in five randomly chosen chromatograms. The limit of detection (LoD) is calculated as the *mean+3*σ

Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
268.58	158.8	23.29	214.9	47.12
152.8	116.1	58.23	181.3	18.75
208.34	48.56	143.4	87.21	51.25
51.3	46.26	105.5	338.80	47.62
546.04	29.48	48.89	766.43	55.57
538.22	48.83	120.0	33.24	37.82
231.03	91.05	56.70	1105	399.8
58.04	21.58	44.06	25.89	12.09
85.39	179.2	756.7	44.33	40.53
274.90	177.8	74.27	156.2	32.24
Mean area = 169.1				
σ = 221.54				
LoD (area) = 664.63 +169.1 = 833.82				

Using a naphthalene standard (20 ppm, peak area 64,694), the calculated limit of detection for naphthalene was 0.0129 ppm.

The two main methods for naphthalene analysis by GC-FID are headspace analysis and direct analysis. Both of these techniques were evaluated to determine the more appropriate technique for this research.

9.2.1 Headspace Analysis

Headspace analysis is a standard method that captures volatile species that have been released from a sample. The technique is so called because it is the volume of vapour above the sample that is analysed. The materials used to capture the volatiles vary, but for this research activated charcoal was used (Bianchi, Careri *et al.*, 2005, Karasali, Balayannis *et al.*, 2006, Wang, Jiang *et al.*, 2006, Borusiewicz and Zieba-Palus, 2007, Reddy, Nelson et al., 2007), .

9.2.1.1 Sample Preparation

A probe was prepared from copper wire (diam. 1 mm, Fisher Scientific UK Ltd.). The copper was cut into strips (3.5cm long). Two thirds of the length of the wire was dipped into sodium silicate solution (Na_4O_4Si Fisher Scientific UK Ltd.); a viscous liquid that forms a glue when heated. The wire was then dipped into activated carbon (300–600 mesh, Fisher Scientific UK Ltd.), which adhered to the sodium silicate. The carbon grain size chosen provided a good adsorptive surface area, without being too fine to block the injection needle. The probe was then placed in a beaker, sealed with Parafilm and placed

in an oven at 58°C for ten minutes to set the glue. To ensure uniformity, several of the probes were prepared simultaneously. A wire cutter was used to bend the wire at 0.5cm from the end, to form a hook from which the wire could hang from the beaker rim.

A headspace glass vial (20ml) was labelled and a test paper sample (4cm x 13cm), contaminated with naphthalene, was rolled up and placed in the vial. An aliquot of *o*-xylene (100µl, 10ppm in carbon disulphide) was added to the vial as the internal standard. A prepared probe was placed in the vial and suspended by hooking over the edge of the vial. The vial was then sealed with Parafilm and placed in the oven for 25 minutes at 58 °C (see section 9.2.1.2). Placing in a dry oven for a period of time encourages the samples to release their volatile species more readily, but it also reduces the effects of relative humidity, which can interfere with adsorption of the charcoal (RI DEM 1997).

After the set time, the samples were removed from the oven and the probe placed into a small vial (1ml) and rinsed with CS_2 (75µl) to extract the adsorbed volatiles from the activated carbon. The injection needle was washed with CS_2 and an aliquot of the sample solution (0.2µl) injected directly into the GC-FID.

9.2.1.2 Oven time optimisation

In order to evaluate the optimal length of time that the probes needed to remain in the oven, six probes were prepared, five of which were placed within a headspace vial containing a weighed and measured sample paper (5cm x 13cm) contaminated with naphthalene, and internal standard (1µl, 7.2ppm). The vial was sealed with Parafilm and placed in the oven (58°C). The sixth probe was analysed immediately. Each of the five probes in the vial was removed systematically every 5 minutes and analysed. The probes were analysed by 'washing' in CS_2 (75µl) and the resulting solution (0.2µl) injected into the GC-FID.

Recent studies into the receptor sites of the surface of activated charcoal have suggested that certain compounds, including aromatic compounds, can bond more readily to the charcoal surface than other compounds. For example, benzene and toluene compete against nitrobenzene, which bonds most readily. It is suggested that pi-pi electron donor-acceptor interactions occur with the aromatic hydrocarbons and the charcoal adsorption sites. It may be possible, therefore, that naphthalene and *o*-xylene compete similarly (Roy, Kongara *et al.,* 1995, Sander and Pignatello, 2005, Zhu and Pignatello, 2005).

The rate of uptake of naphthalene and *o*-xylene by the activated charcoal probes is illustrated in Figs. 58 and 59, respectively. The amount of naphthalene adsorbed by the probes increased steadily over time. Unfortunately, saturation was not achieved, and hence a true optimisation of time for naphthalene uptake not determined. The adsorption curve for *o*-xylene over the same time period was shown to be different than for naphthalene, and no significant increase in adsorption over time observed.

An appropriate time for the vials to be left in the oven to ensure a good uptake of naphthalene was

considered to be 25 minutes.

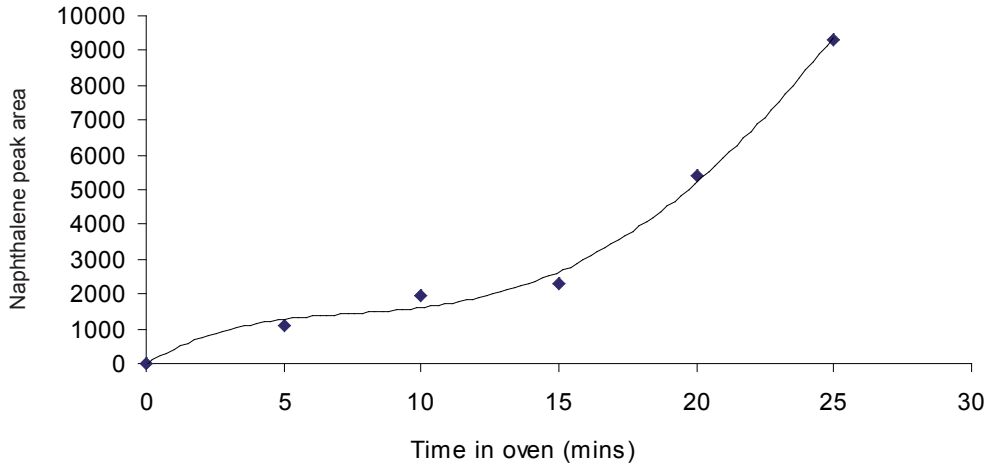

Figure 58 The rate of naphthalene uptake by the activated charcoal probes in a sealed vial within an oven (58°C). Saturation was not achieved.

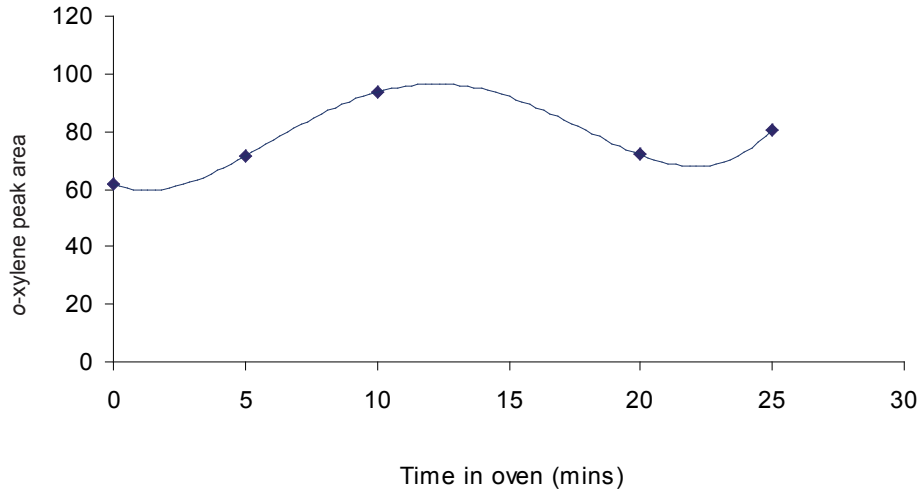

Figure 59 The rate of *o*-xylene uptake by the activated charcoal probes in a sealed vial within an oven (58°C).

9.2.1.3 Evaluation of the Errors Incurred

Pipetting small volumes of volatile liquids

As small volumes of volatile liquids had to be pipetted during the headspace analysis, it was important to establish the reproducibility of the action. A series of volumes were pipetted into a glass vial and weighed immediately using an analytical balance. Each volume was pipetted 10 times (Table 38). Both water and a CS_2/naphthalene mixture were evaluated.

Table 38 Reproducibility of micropipetting a range of volumes of CS_2/naphthalene mixtures (C+N)

Measurement	C+N 1ml	C+N 50µl	C+N 5µl	C+N 1µl	Water 1ml	Water 50µl	Water 20µl
1	1.138	0.045	0.003	0.0012	1.0074	0.0510	0.0210
2	1.182	0.054	0.004	0.0014	1.0081	0.0509	0.0199
3	1.191	0.052	0.003	0.0012	1.0094	0.0502	0.0202
4	1.177	0.054	0.003	0.0012	1.0094	0.0505	0.0205
5	1.182	0.048	0.002	0.0014	1.0079	0.0504	0.0204
6	1.176	0.063	0.003	0.0020	1.0081	0.0504	0.0204
7	1.178	0.054	0.004	0.0020	1.0077	0.0505	0.0205
8	1.139	0.049	0.004	0.0018	1.0070	0.0502	0.0202
9	1.181	0.053	0.004	0.0016	1.0070	0.0504	0.0204
10	1.177	0.061	0.003	0.0020	1.0064	0.0502	0.0202
Mean	1.172	0.0533	0.003	0.0016	1.0078	0.0505	0.0204
± RSD (%)	1.555	10.32	20.45	21.88	0.098	0.553	1.409

The errors incurred whilst pipetting the volatile mixture of CS_2 and naphthalene were significant (up to ± 21%), and much higher than resulted from pipetting water. This raised questions about the reliability of the internal standard addition.

Reproducibility of *o*-xylene standard additions

To determine whether the internal standard addition (100µl *o*-xylene, 10ppm) was giving reproducible peak areas, 20 chromatograms from a range of standard solution analyses were compared. The *o*-xylene peak areas are given in Table 39. The variability of the peak areas were found to be within 8% and in agreement with the data in Table 38 above.

Table 39 Variability of the *o*-xylene peak areas following standard additions to a range of naphthalene solutions

Analysis	*o*-xylene peak area	Analysis	*o*-xylene peak area
1	35041	11	35389
2	32112	12	32176
3	32485	13	31784
4	32272	14	30949
5	30701	15	33381
6	35992	16	32620
7	32132	17	27876
8	31638	18	31432
9	32608	19	25225
10	28513	20	30519
Mean peak area = 31742 ± 2502 (8%)			

Efficiency of headspace extraction

In order to determine the efficiency of the extraction and head space analysis, nineteen naphthalene standard solutions, over a range of concentrations, were subjected to the extraction procedure and analysed by GC-FID. The results (Table 40) show the extraction procedure to be very inefficient, with a mean extraction efficiency of only 1.2%, significantly limiting the sensitivity of the method. The small peaks observed will potentially lead to significant errors being incurred during peak integration (Table 41). For naphthalene concentrations of 1–20 ppm, the errors incurred through peak integration will range from 26–62%.

Table 40 The extraction efficiencies of the head space method for naphthalene from a range of standard solutions

Actual Concentration (ppm)	*o*-Xylene	Naphthalene	Peak Area Ratio (Naphthalene: *o*-xylene)	Measured Concentration (ppm)	Efficiency of extraction (%)
0.79	152096.000	553.000	0.004	0.031	3.900
1.3	1780.916	5.132	0.003	0.023	1.800
2.5	2200.890	13.525	0.006	0.046	1.800
2.5	1870.840	9.790	0.005	0.038	1.500
5	2543.012	12.919	0.005	0.038	0.760
7.94	525902.000	14785.000	0.028	0.214	2.700
13	981.905	11.958	0.012	0.093	0.717
13	1238.921	13.836	0.011	0.084	0.650
23.8	274742.000	26235.000	0.096	0.727	3.100
25	2935.180	71.044	0.024	0.184	0.740
63	83.744	3.096	0.037	0.283	0.450
63	174.481	6.105	0.035	0.268	0.430
80	440663.410	44995.650	0.102	0.252	0.250
127	118.899	4.881	0.041	0.314	0.250
199	127095.000	34476.000	0.271	2.075	1.000
318	112.545	22.278	0.198	1.516	0.480
397	736615.000	429855.000	0.583	4.472	1.100
794	132232.000	159161.000	1.204	9.219	1.200
Mean efficiency of extraction (%) = 1.27 ± 1.0 (85%)					

Table 41 Errors from peak integration for a range of naphthalene solutions using head space analysis

Concentration (ppm)	Naphthalene peak areas (n)	Counting error (√n)	Error (%)
1	2.62	1.6	62
2	6.24	2.5	40
3	11.57	3.4	29
6	14.85	3.9	26
8	10.31	3.2	31
20	13.62	3.7	27
		Mean error	36%

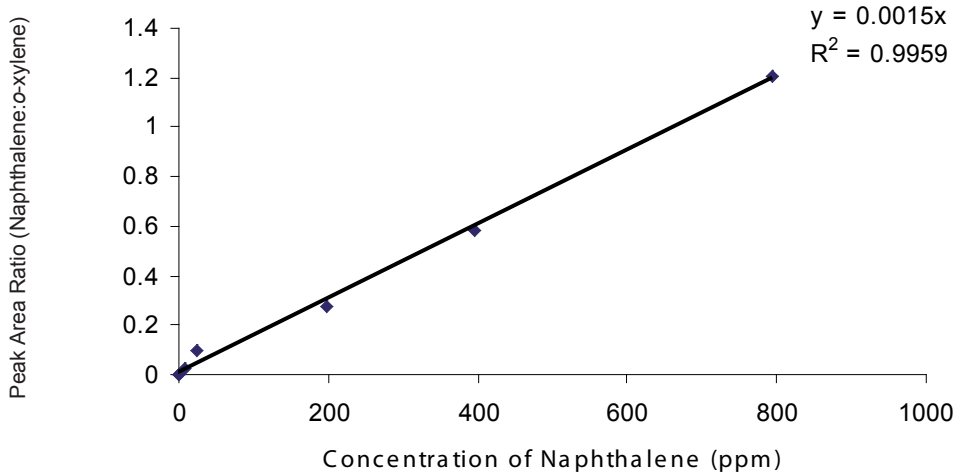

Figure 60 Probe analysis calibration curve for standards.

Reproducibility of the headspace analysis

Replicate analyses of standard naphthalene solutions (900µl, 1.3–25ppm, 100µl *o*-xylene, 10 ppm) were used to evaluate the reproducibility of the head-space technique (Table 42). In general, the reproducibility was poor, with relative standard deviations ranging from ± 8% to ± 33% (mean rsd = ± 14%). Furthermore, the calibration curve (Fig. 61) shows a poor relationship between peak ratio and concentration ($r^2 = 0.9148$). The poor reliability of this method is only partly due to the uncertainty in the standard additions (Fig.62).

Table 42 Reproducibility of headspace analysis for a series of naphthalene solutions

ppm	n	Mean Peak Area ratio	± RSD(%)
1.3	4	0.002656	12
1.6	3	0.005232	10
2.4	3	0.005567	16
2.5	5	0.00563	8
4	3	0.007454	9
5	3	0.005788	9
13	4	0.011012	11
25	4	0.0391	33

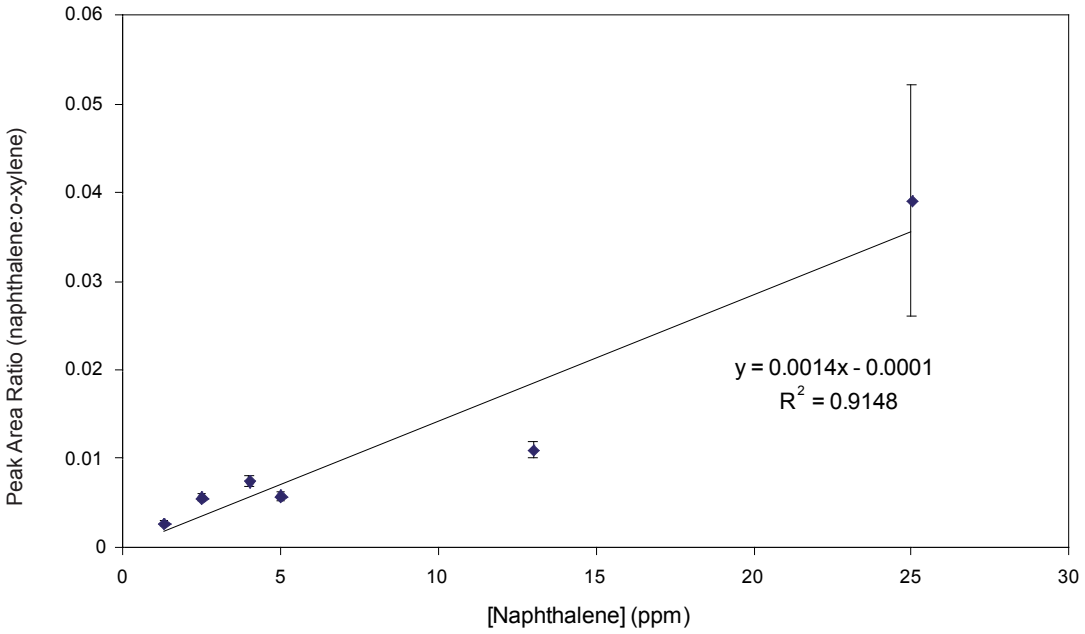

Figure 61 Reliability of the head-space analysis method using *o*-xylene as internal standard. The relationship between peak area ratio and naphthalene concentration is weak.

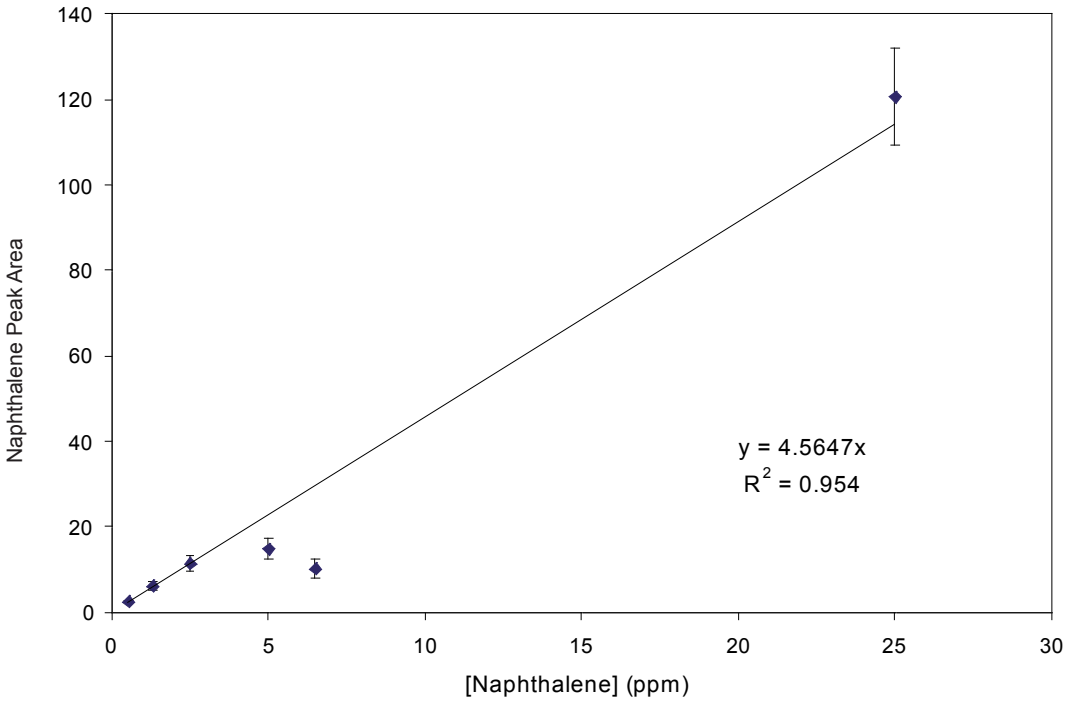

Figure 62 The relationship between naphthalene peak area and naphthalene concentration using the head space method is slightly better without the internal standard addition

Estimation of errors incurred through headspace analysis

The main errors incurred through the headspace analysis were through pipetting the volatile liquids (± 8%) and the peak integration (± 36%) resulting from the poor efficiency of the technique. The combined error is calculated as 37%, higher than indicated by the reproducibility measurements, which suggested an overall error of 8–33% over the same concentration range.

9.2.2 Direct Analysis

Direct analysis involves the analyte being solvent-extracted directly from the sample and injected into the GC-FID.

9.2.2.1 Method Evaluation

Standards were made up from 0-25mg/L of naphthalene in CS_2 and each was injected (1.0µl) into the GC-FID (Fig 63).

Eighteen test samples were prepared (5mm x 132mm) doped with a range of naphthalene concentrations (0, 5, 10, 15, 20 and 25mg/L). Each sample was cut into three small squares (5mm x 5mm) and each placed into vials (20ml). Internal standard (*o*-xylene, 500µl, 10ppm) was added. An aliquot of CS_2 (4.5ml) was added to the vial, ensuring the paper was covered. The vial was then sealed with Parafilm and placed in a sonic bath for 90 seconds. The sample solution (1.0µl) was injected directly into the GC-FID, and the naphthalene concentration determined to evaluate the method.

Figure 60 shows the naphthalene: *o*-xylene peak area ratios are highly correlated to the known naphthalene concentrations (r^2=0.995). The reproducibility was relatively good (rsd= ±0.4–6.5%). The variation observed in some of the samples is likely to be a result of the doping procedure leading to an inhomogeneous distribution, rather than an inherent weakness in the methodology.

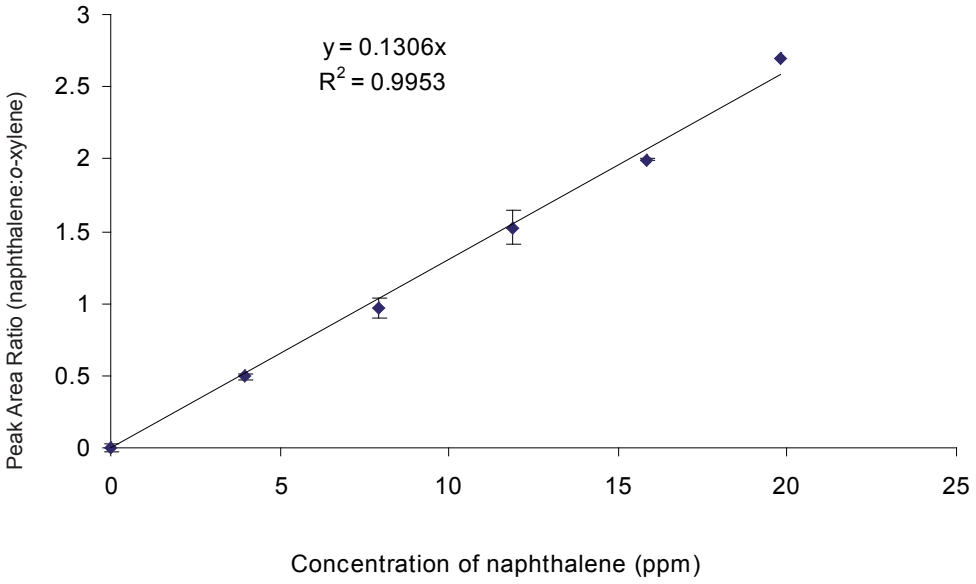

Figure 63 The correlation between the measured peak area ratios (naphthalene:*o*-xylene) for the doped test samples (n=3) shows good correlation with the known concentrations, indicating the direct method works well.

9.2.2.2 Optimising the Sonication Time

The purpose of the sonic bath is to efficiently remove the naphthalene from the herbarium paper samples in the shortest possible time, thus maximising the extraction process. The optimal time for sonication was therefore determined.

A piece of sample paper (5mm x 132mm), contaminated with naphthalene, was weighed and cut into small pieces (25mm^2). Five test samples were then placed into vials (20ml). Internal standard (*o*-xylene, 500µl, 10ppm) was added. An aliquot of CS_2 (4.5ml) was added to the vial, ensuring the paper was covered. The vials were then sealed with Parafilm and placed in a sonic bath for 0, 30, 90, 180 and 220 seconds, respectively. The sample solution (0.2µl) from each vial was injected directly into the GC-FID, and the naphthalene concentration determined.

The concentration of naphthalene extracted into the CS_2 was at a maximum after 120 seconds of sonication (Fig.64).

The optimum time for sonication was determined to be 120 seconds.

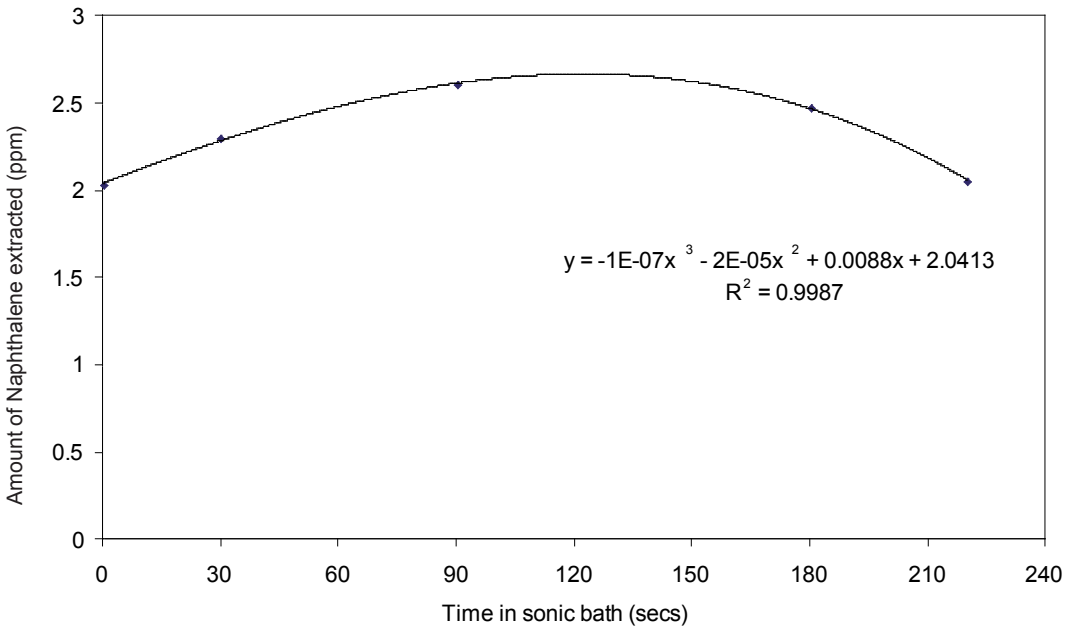

Figure 64 The effect of changing sonication time on the amount of naphthalene extracted by CS_2. Maximum extraction occurred after 120 seconds of sonication.

9.2.2.3 Evaluation of the Errors Incurred

Reproducibility in pipetting small volumes of volatile liquids

As with the headspace analysis, the addition of small volumes of the volatile o-xylene will lead to errors in the measured concentrations. A larger volume of internal standard (500µl) was used for the direct analysis, leading to better reproducibility than observed in the headspace analysis. It is estimated that internal standard addition is achieved to within 4% reproducibility.

Peak integration

The errors incurred through peak integration are estimated in Table 43. For naphthalene concentrations of 0.5–30 ppm, the expected errors will be 0.3–3%.

Table 43 Errors from peak integration for a range of naphthalene solutions using direct analysis

Concentration (ppm)	Naphthalene peak areas (n)	Counting error (√n)	Error (%)
0.5	985	31.4	3.19
6	18552	136.2	0.73
12	34925	186.9	0.54
18	51991	228.0	0.44
25	68822	262.3	0.38
30	89230	298.7	0.33
		Mean error	0.9%

Reproducibility of naphthalene analysis

To determine the reproducibility of the naphthalene analysis by the direct method, replicate sections 7 and 10 from sample D1 were tested repeatedly using the exact same method each time (see section 9.2.2.1) (Table 44).

In general the reproducibility was good (Table 45), with the relative standard deviations varying from ± 0.5 to ± 8.6% (mean rsd = ± 3.6%).

Table 44 Samples taken in quadruple and triplicate of naphthalene map to determine accuracy in methodology. Equation y=0.1287x

Sample No	N/o-x ratio	Dilution	Paper wt (g)	Area cm^2	Naphthalene (µg/g)	Naphthalene (µg/cm^2)
10	0.091	8	1.19	115	4.753	0.049
10	0.090	8	1.19	115	4.701	0.048
10	0.089	8	1.19	115	4.649	0.048
Mean					4.701	0.048
Std					0.052	0.001
Coefficient of variance %					1.11	2.08
7	0.088	8	1.13	105	4.841	0.052
7	0.083	8	1.13	105	4.566	0.049
7	0.085	8	1.13	105	4.676	0.05
Mean					4.694	0.050
Std					0.138	0.002
Coefficient of variance %					2.94	4.0

Table 45 Replicate analyses for naphthalene using direct techniques to determine precision in methodology.

ppm	n	± RSD(%)
4	3	2.6
8	3	4.9
12	3	0.5
16	3	0.6
20	3	8.6
G	3	5.8
J	4	2.2

Estimation of errors occurred through direct analysis

The main errors incurred through the direct analysis were through pipetting the volatile liquids (± 4%) and the peak integration (± 0.9%). The error associated with the determined concentration of naphthalene from the direct analysis can, therefore, be estimated to be in the region of ± 4.1%. This is in good agreement with the reproducibility studies, which suggested an overall error of 0.5– 8.6% over the same concentration range.

Comparing the headspace and direct analysis methodologies

Although the two methods have been shown to be valid for the analysis of naphthalene at the levels observed on the herbarium sheets, clearly the direct analysis is a more efficient, more precise and more sensitive method. Peak areas observed for standard analysis are significantly higher for the direct analysis than the headspace analysis (2890–57796, compared to 2.6–13.6, respectively, over the concentration range 1–20 ppm). This has led to significantly smaller errors being incurred from peak integration using the direct method (0.3–3%, as compared to 26–62% from the headspace analysis). This is reflected in the observed mean reproducibility's of the two methods: ± 3.6% and 14% for the direct and headspace analysis, respectively. The direct method was, therefore, used for this research.

9.2.3 Method for analysis of naphthalene on herbarium sheets

The method adopted for the analysis of naphthalene on herbarium sheets is given below:
Samples from the herbarium sheet (5mm x 132mm) were taken and cut into three small squares (5mm x 5mm), each of which was placed into a vial (20ml). Internal standard (*o*-xylene, 500µl, 10ppm) was applied to each sample paper, and an aliquot of CS_2 (4.5ml) added to the vial, ensuring the sample paper was covered. The vial was then sealed with Parafilm and placed in a sonic bath for 120 seconds. The sample solution (0.2µl) was injected directly into the GC-FID, and the naphthalene concentration determined.

The analytical conditions for the GC-FID are summarised in Table 46.

Table 46 Instrument and method settings for GC-FID analysis of naphthalene

GC-FID	**Varian 3800**
Internal standard	*o*-xylene
Solvent	carbon disulfide
Injection temperature	200°C
Iso time (min)	2
Detector temperature	300°C
Initial oven temperature	40°C
Ramp rate	10 °C-110°C, and 30°C-300°C
Final temperature	300°C
Total run time	15.33mins
Detector	FID
Carrier gas	Helium
Sample volume	0.2µl
Split ratio	0
Flow rate	20ml/min
Column	DB1MS
Split bottom	No
Purge top	Yes

9.3 Naphthalene Decontamination

9.3.1 Sample Preparation

Fifteen herbarium sheets were used for the decontamination studies (Table 36). The sampling procedure for the herbarium sheets is illustrated in Figure 65. Each herbarium sheet was cut into ten sections (approximately 132mm x 84mm. Five sections (S1-S5) were used for testing, whilst the other five sections (C1-C5) were used as controls. Once prepared, the control samples were stored separately in gas tight (Archipress®) bags.

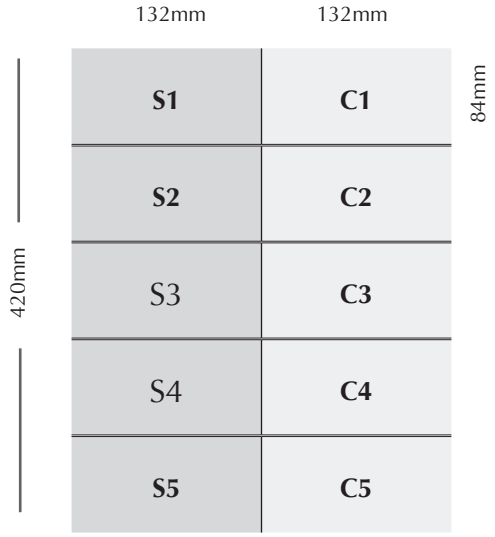

Figure 65 Sampling procedure for herbarium sheets for the naphthalene decontamination studies. Sections S1-S5 were used for testing and sections C1-C5 were controls.

9.3.2 Naphthalene Distribution

In order to determine whether the naphthalene was homogeneously distributed across an entire herbarium sheet, the concentration of naphthalene was measured in all ten sections of sheets D1-D3 (See Table 36).

9.3.3 Decontamination Tests

9.3.3.1 Freeze-drying

The samples for testing (Table 36) were placed into a Super Modulyo Edwardes freeze-dryer that freezes to -40°C. After 1 hour, the vacuum pump was switched on and the samples were freeze-dried for 2, 7, 25, 48 and 72 hours. After the required time, the vacuum pump was switched off and the air released from the chamber. The sample was taken out of the freeze drier and a section (84mm x 132mm) of sample was removed and placed into a separate Archipress® bags, each sample was measured, weighed and labelled. The remainder of the sample was placed back inside the freeze-dryer, which was switched back on and left for the next time period. After completion of the experiment, all samples and controls were analysed and the naphthalene content determined.

9.3.3.2 Oven Heating

Test samples were placed in a box within an oven (a UniTemp Laboratory oven with an internal thermometer fitted) for 120 hrs at 40°C with uncontrolled relative humidity. Samples were left in the oven for 1, 3, 6, 24, 48 and 72 hours. After the required time, the sample was taken out of the oven and a section of paper (84mm x 132mm), was removed, weighed, measured and labelled and placed in a sealed Archipress® bag. The remaining paper sample was then returned to the oven. On completion of the experiment, test and control samples were analysed and the naphthalene content determined.

9.3.3.3 Air Drying

Test samples were placed in a fume cupboard (Safe Lab Systems™ Airone Blue fume cupboard with C100 Size B charcoal filter) and left to air for 8, 24, 48 and 72 hours. The fume cupboard was left running for 8 hours a day, but at night it was switched off. After the required time, the samples were taken out of the fume cupboard and a section (84mm x 132mm) of sample was removed. The remaining sample was then returned to the fume cupboard. The removed sections were weighed and measured and kept securely within a gas tight Archipress® bag. On completion of the experiment, test and control samples were analysed and the naphthalene content determined.

10 Results

10.1 Naphthalene Distribution

The distribution maps for herbarium sheets D1, D2 and D3 are given in Figure 66.

	5.3	4.3	4.9	2.2	0.35	0.36
	8.3	6.6	4.9	1.6	0.42	0.25
	4.6	5.6	5.2	2.6	0.45	0.34
	5.1	5.8	nd	nd	0.56	nd
	5.6	4.4	nd	nd	nd	nd
∑	28.9	26.7	15.0	6.4	1.78	0.95
MEAN	5.8	5.3	5.0	2.1	0.45	0.32
RSD%	25	18	3.5	24	20	19

Figure 66 Distribution of naphthalene across herbarium sheets D1, D2 and D3 (left to right). The total sum, mean and relative standard deviations of naphthalene concentrations for the left hand side and right hand side is also given.

10.2 Naphthalene Decontamination

The results of the decontamination tests for air-drying, freeze-drying and oven-heating are given in Tables 47-49, respectively.

Table 47 Naphthalene concentrations (ppm) determined for test and control samples using the air-drying decontamination technique

Time (hours)	[Naphthalene] (ppm) Herbarium sheet A1		[Naphthalene] (ppm) Herbarium sheet A2		[Naphthalene] (ppm) Herbarium sheet A3	
	Test	Control	Test	Control	Test	Control
8	7.4	9.7	8.9	15.4	6.9	13.3
23.5	7.8	13.5	9.6	19.5	6.2	13.9
48	3.4	11.9	10.4	17.0	3.11	14.7
72	4.4	9.1	13.5	16.4	6.6	17.2
∑	23.0	44.2	42.4	68.3	22.8	59.1
Mean	5.8	11.1	10.6	17.1	4.0	14.8

Table 48 Naphthalene concentrations (ppm) determined for test and control samples using the freeze-drying decontamination technique

Time (hours)	[Naphthalene] (ppm) Herbarium sheet F1		[Naphthalene] (ppm) Herbarium sheet F2		[Naphthalene] (ppm) Herbarium sheet F3		[Naphthalene] (ppm) Herbarium sheet F4	
	Test	Control	Test	Control	Test	Control	Test	Control
2	18.4	27.3	18.4	31.0	19.7	16.5	24.1	21.2
7	25.4	21.2	27.3	29.7	8.9	24.1	20.0	10.3
25.5	24.8	22.0	24.3	26.4	6.7	25.1	19.6	17.9
48	24.3	40.1	28.0	10.6	11.1	25.9	19.2	16.6
72	28.0	24.1	16.1	9.2	5.6	25.3	17.3	24.0
∑	120.9	134.7	114.1	106.9	52.0	116.9	100.2	90.0
Mean	24.2	27.0	22.8	21.4	10.4	23.4	20.0	18.0

Table 49 Naphthalene concentrations (ppm) determined for test and control samples using the warm oven decontamination technique

Time (hours)	[Naphthalene] (ppm) Herbarium sheet H1		[Naphthalene] (ppm) Herbarium sheet H2		[Naphthalene] (ppm) Herbarium sheet H3		[Naphthalene] (ppm) Herbarium sheet H4	
	Test	Control	Test	Control	Test	Control	Test	Control
1	16.3	26.3	15.2	17.6	13.8	15.7	16.9	20.9
3	16.1	27.6	11.8	27.6	4.5	15.6	17.6	27.5
6	30.4	34.6	16.5	31.5	3.7	18.6	13.2	19.3
24	14.0	30.6	10.8	30.4	11.4	14.0	12.5	18.8
48	24.7	26.3	16.4	31.7	9.7	12.5	16.1	23.3
72	18.7	24.2	8.8	30.6	8.4	9.9	10.0	29.5
∑	120.2	169.6	79.5	169.4	51.5	86.3	86.3	139.3
Mean	20.0	28.3	13.3	28.2	8.6	14.4	14.4	23.2

Table 50 Naphthalene concentrations across all air-dried sheets, showing total loss (ppm) and % loss.

Time (hours)	[Naphthalene] (ppm) ∑ Test A1, A2, A3	[Naphthalene] (ppm) ∑Control A1, A2, A3	[Naphthalene] (ppm) Total loss
8	23.15	37.71	14.56
23.5	19.53	45.95	26.42
48	16.92	42.85	25.93
72	24.56	41.97	17.42
∑	84.16	168.49	84.33
Mean	21.04	42.12	21.08
% Loss	50.05		

Table 51 Naphthalene concentrations across all freeze-dried sheets, showing total loss (ppm) and % loss.

Time (hours)	[Naphthalene] (ppm) ∑ Test F1, F2, F3, F4	[Naphthalene] (ppm) ∑Control F1, F2, F3, F4	[Naphthalene] (ppm) Total loss
2	80.57	37.71	14.56
7	82.05	45.95	26.42
25.5	75.41	42.85	25.93
48	82.69	41.97	17.42
72	67.05	168.49	84.33
∑	387.77	413.09	25.32
Mean	77.55	82.62	5.06
% Loss	6.13		

Table 52 Naphthalene concentrations across all oven-heated sheets, showing total loss (ppm) and % loss.

Time (hours)	[Naphthalene] (ppm) ∑ Test H1, H2, H3, H4	[Naphthalene] (ppm) ∑Control H1, H2, H3, H4	[Naphthalene] (ppm) Total loss
1	62.18	74.87	12.69
3	49.91	91.41	41.5
6	63.72	96.76	33.04
24	48.64	87.17	38.53
48	66.86	87.17	20.31
72	45.75	87.57	41.82
∑	77.55	82.62	187.89
Mean	56.18	87.49	31.32
% Loss	35.79		

11 Discussion

Naphthalene is a particularly volatile molecule and has proved difficult to quantify at the trace level. Nevertheless, the methodology developed minimised the analytical error to around ±9%. This was considered a good precision for the trace quantities involved.

It was expected that the overall distribution of naphthalene across the specimen sheets would be fairly uniform, since naphthalene was not directly applied to the specimen, but absorbed from the cabinet or drawer environment. The air-borne naphthalene concentration in Cabinet 57, where all of the samples were stored, was determined to be 0.5-0.62mg/m^3. The specimens had been stored in this environment for a long time and hence a significant amount of naphthalene would have been absorbed into the cellulose matrix.

Analysis of specimen sheets D1–3 (Table 53) showed that the distribution of naphthalene was not uniform. Variation of up to ±45% was observed (D2), significantly higher than expected from the analytical uncertainty. On closer inspection of the D2 results, however, there is a significant difference between the left- and right-hand sides of the specimen sheet, with mean concentrations of 5.0 and 2.1 ppm, respectively, suggesting that some other factor is involved. Sample sheet D2, therefore, may not be a representative sample. Sample sheets D1 and D3 showed a better uniformity, although still not good, with variation in the region of 20%. Comparison of the test half (left) with the control half (right), showed the mean naphthalene concentrations to be comparable, with similar uncertainties (although still high, at ±20%).

Table 53 Variability in naphthalene concentration across herbarium sheets, measured as a relative standard deviation from the mean

Sheet	**Mean naphthalene concentration (ppm) ± rsd (%)**		
	Overall (n=10)	Test (LHS) (n=5)	Control (RHS) (n=5)
D1	5.6 ± 21	5.8 ± 25	5.3 ± 18
D2	3.6 ± 45	5.0 ± 3.5	2.1 ± 24
D3	0.39 ± 25	0.45 ± 20	0.32 ± 19

The non-uniformity in naphthalene concentrations across the sheets, presents a number of problems in interpreting the results of the decontamination tests. Due to the destructive nature of the analytical methodology, a ‘before’ and ‘after’ analysis was not possible on the same sample. With the evident inhomogeneity, the control samples may not adequately represent the test samples, even though they are from the same specimen sheet.

The effectiveness of air-drying in removing naphthalene was tested on specimen sheets A1–A3 (Figure 67). Whereas sample sheets A1 and A3 behaved in a similar manner, sample sheet A2 was a little

different. All three sample sheets lost significant amounts of naphthalene during the air-drying process. Sample sheets A1 and A3 lost more than 70% of the naphthalene in the first 48 hours (71 and 79%, respectively), before reabsorption began to dominate. Sample sheet A2 lost 51% in the first 23.5 hours and then began to absorb naphthalene again. All three sample sheets lost naphthalene most rapidly during the first few hours of the air-drying process. This may reflect the loss of surface naphthalene, with the more deeply absorbed naphthalene being lost over time. The observed reabsorption of naphthalene at 23.5 and 48 hours into the process is surprising, since the desorbed naphthalene was removed rapidly by the air flowing over the samples. This apparent reabsorption does not coincide with the fume-hoods being switched off overnight. It is, however, possible that naphthalene was being drawn to the surface of the specimen sheets from deep within the fibres by the air-drying process. This would increase the surface concentration and give the impression that naphthalene was being reabsorbed (this assumes that the solvent extraction carried out prior to analysis removed only the surface naphthalene).

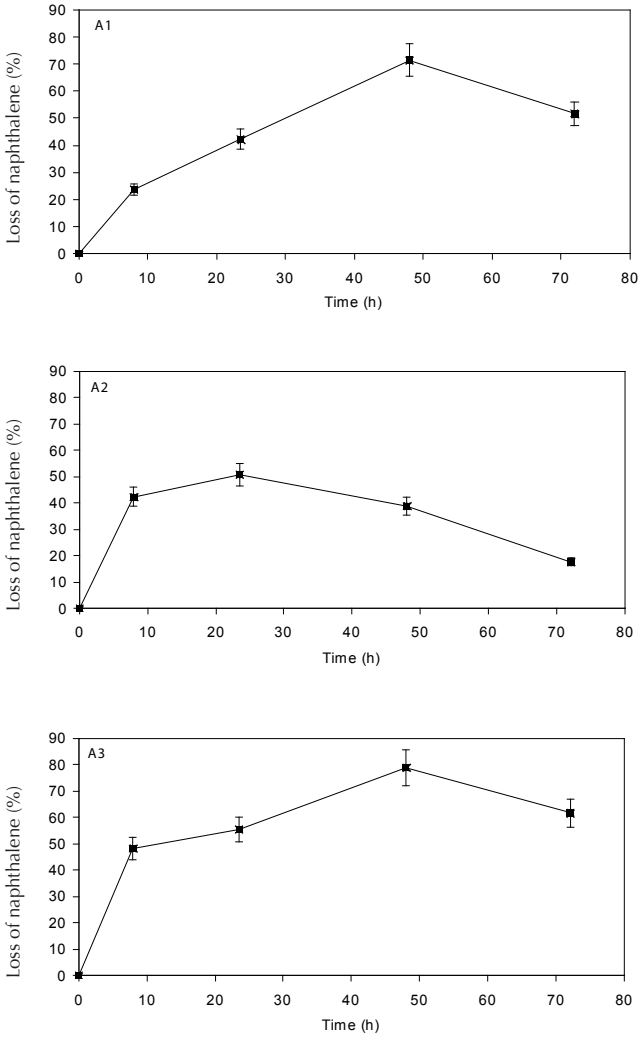

Figure 67 Loss of naphthalene over time during the air dried tests with samples A1–A3.

Although sample sheets A1 and A3 had similar loss characteristics, the rate and extent of naphthalene loss from sample A3 was significantly greater within the first 24 hours (Figure 68). It is apparent, therefore, that sample sheet A3 does not hold on to the naphthalene as strongly as A1, indicating a much lighter-weight paper. Sample sheet A1 is a thick, finished paper (149 g/m^2), whereas A3 is a thin, coarse paper (85 g/m^2).

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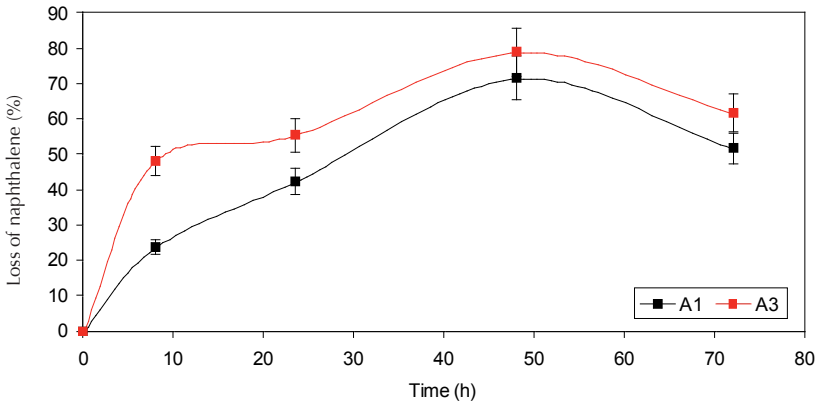

Figure 68 Comparison of the rates of naphthalene loss between sample sheets A1 and A3

Overall, the air-drying process was fairly effective at removing the naphthalene from the specimen sheets.

In simple terms, the freeze-drying process will be at its most effective when there is a good differential between the vapour pressure of the chemical being removed and the partial pressure of the vapour surrounding the sample. It is relatively easy to get the correct conditions for the removal of water, with a vapour pressure of 15.5 mmHg at 20°C, but hydrocarbons are notoriously difficult to remove completely (Taylor, 2006, Connell, 2006). Research into the use of freeze-drying to remove organochlorine pesticide residues (lindane, dieldrin and DDT) were unsuccessful, due to the low vapour pressures of the residues (9.4x10^{-6}, 1.8x10^{-7}and 1.5x10^{-3} mmHg at 20°C, respectively) and the trace quantities present (Zabik and Dugan 1971). Although naphthalene has a high vapour pressure for a solid, in comparison with water, it is very low and approaching that of DDT (5.3x10^{-2} mmHg at 20°C).

Specimen sheets F1–F4 all showed a cyclic behaviour during the 72 hours in the freeze-dryer (Figure 69), although the behaviour of each was different. Specimen sheet F1 showed an initial rapid release of naphthalene (33% loss) in the first 2 hours and then a rapid reabsorption over the next 5 hours.

A slow release of naphthalene was again observed, reaching a maximum loss (39%) at 48 hours. Interestingly, specimen sheet F2 was absorbing significant amounts of naphthalene (up to a 164% gain) at the same time that F1 was off-gassing. As there were up to 9 specimen sheets in the freeze-drying cabinet at any one time, it is possible that the naphthalene was not being effectively removed from the cabinet environment and was being reabsorbed by the samples present.

Both sample sheets F1 and F2 are light-weight papers: F1 is a thin, coarse paper (121 g/m^2), whilst F2 is a lighter, cotton paper (118 g/m^2). Both of these papers would be expected to release naphthalene readily. During the first two hours of freeze-drying, both F1 and F2 showed a rapid rate of naphthalene release, leading to a 33% and 41% loss, respectively.

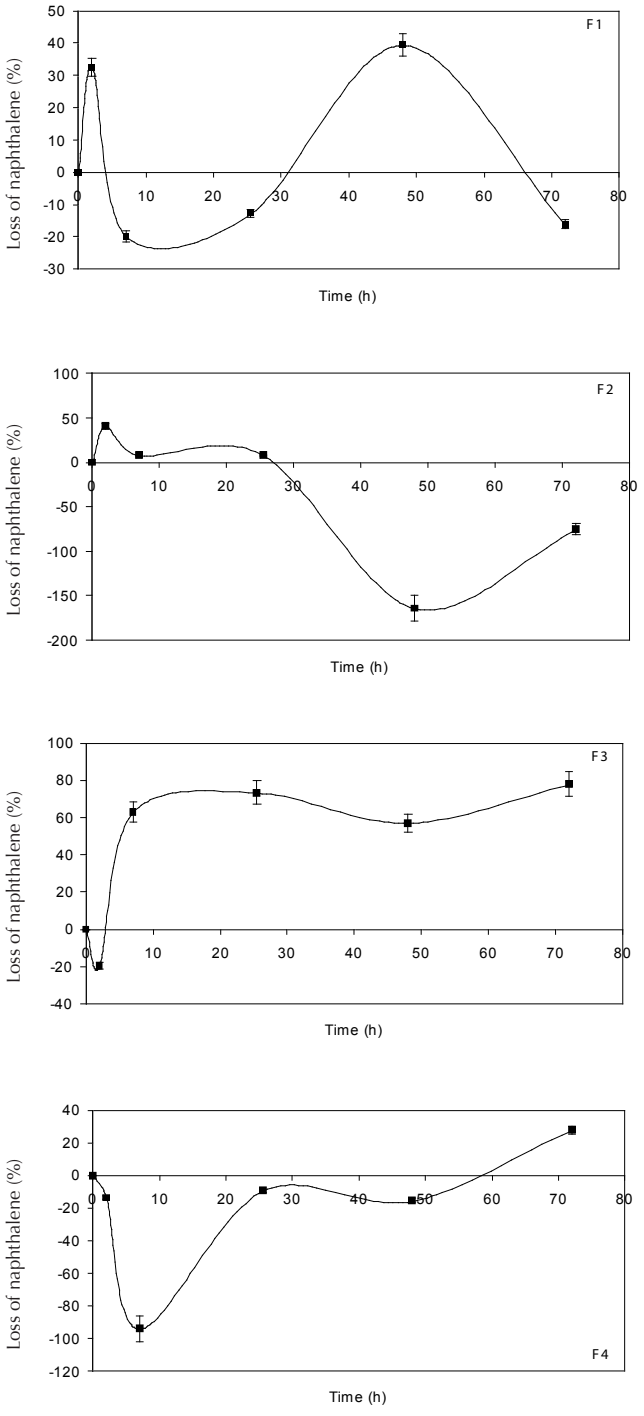

Figure 69 Loss of naphthalene over time during the freeze dried tests with samples F1–F4.

Specimen sheet F3 is a thick, unfinished, paper (165 g/m^2) with an open, porous structure. Its porous nature allows naphthalene to be readily absorbed from the environment. This was observed during the first 2 hours of the test, when the sample increased its naphthalene content by 19%. Over the next 5 hours (t=2–7h), whilst the other papers were absorbing naphthalene, sheet F3 was rapidly releasing it. A total naphthalene loss of 82% was recorded during this period.

Specimen sheet F4 also gained naphthalene in the first 2 hours of freeze-drying. When sheet F3 began to rapidly release naphthalene (t=2–7 hours), F4 continued to absorb it, achieving a maximum gain (94%) after 7 hours. In general, over the remaining time in the freeze-dryer, sample F4 slowly released the naphthalene, achieving an overall loss (28%) after 72 hours. Sheet F4 is a thick, finished paper (131 g/m^2), with a smooth non-porous texture resulting from being sized with either gelatine or rosin. The presence of the size may well affect the rate of naphthalene release from the paper.
Overall, the freeze-drying was only partially successful at removing the naphthalene and seemed to be dependent upon the paper type. The excessive reabsorption observed in three of the samples would be a concern if this technique was utilised.
The cyclic behaviour of the specimen sheets during freeze-drying was also observed in specimen sheets H1–H4 during the oven-drying tests (Figure 70). This is likely to be due to naphthalene remaining in the oven environment and being reabsorbed over time.

Specimen sheet H1 had the highest initial rate of naphthalene loss of the four sheets studied, losing 38% during the first hour. The other sheets showed much slower rates of naphthalene loss during this time (14%, 12% and 19% for H2, H3 and H4, respectively). During the remainder of the time in the oven, the sheet absorbed and desorbed naphthalene (maximum=54%; minimum= 6%) in slow cycles, before achieving an overall loss of 23% after 72 hours. Sheet H1 was a heavy, finished paper (128 g/m^2), which appeared to be made from cotton. The surface of the paper was coated, giving a hard and shiny surface, which on wetting, peeled off like glue. This coating was separate to size and may have acted as a barrier to the absorption and desorption of naphthalene from the body of the paper. The rapid release during the first hour of the test may have reflected loss of the naphthalene lying on the surface of this hard, shiny coating.

Specimen sheet H2 showed an initial rapid loss of naphthalene over the first 3 hours of oven-drying, achieving a loss of 57% during this time. For the remainder of the time in the oven, the sheet slowly absorbed and desorbed naphthalene to a much smaller extent than observed with H1. After 72 hours, a naphthalene loss of 71% was observed. Specimen sheet H2 was a thin, unfinished, paper with a coarse and porous surface (90 g/m^2). Although it did not lose naphthalene as quickly as H1 in the first hour, overall the naphthalene was lost more readily and less inclined to be reabsorbed.

Specimen sheet H3 appeared to lose surface naphthalene very rapidly during the first 3 hours, losing 71% during this time. After 6 hours, however, the sheet absorbed naphthalene readily, achieving only a 15% net loss of naphthalene after 72 hours. Specimen sheet H3 is a thick white card with a finished surface (155 g/m^2) and overall appeared inclined to favour the absorption of naphthalene rather than release.

Of the four specimen sheets studied, sheet H4 showed the least tendency to reabsorb naphthalene during the 72 hours of oven-drying. A rapid loss of naphthalene occurred during the first 3 hours (36%), followed by a more gradual release over the next 69 hours. The specimen sheet achieved an overall 66% loss of naphthalene in 72 hours. H4 was a thick, finished paper (138 g/m^2).

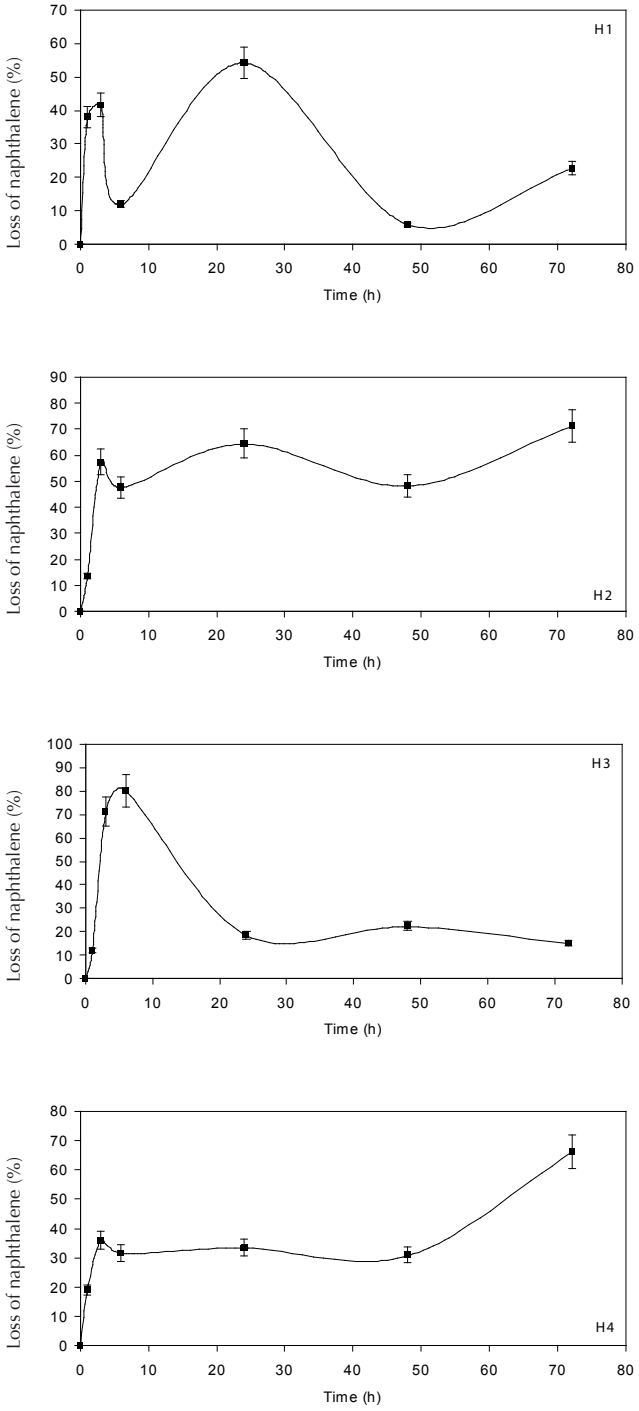

Figure 70 Loss of naphthalene over time during the oven dried tests with samples H1–H4.

On the whole, the tests have shown that air-drying was more efficient at removing naphthalene than either freeze-drying or oven-drying. There was some evidence to suggest that the naphthalene held within the fibres in the body of the paper moved to the surface during the drying process and that longer periods of time may be required to reduce the overall naphthalene concentration in the specimen sheets.

The apparent cycles of naphthalene desorption / reabsorption present in the freeze-drying and oven-drying tests were probably due to the desorbed naphthalene not being removed from the environment and remaining available for reabsorption. Movement of naphthalene from the body of the paper to the surface during the drying may have contributed to the increased surface concentrations observed.

The influence of paper type on the efficiency of naphthalene removal was evident. The thin, unfinished and hence porous, papers were much more efficient at losing naphthalene than the heavier, finished and coated papers. Their porous structure allows the naphthalene to remain more mobile.

The method of air-drying is not only the most effective method for the removal of naphthalene, but also the most cost-effective of the three methods tested and the least damaging to the specimen.

Freeze-drying is carried out at temperatures in the region of -40^{o}C (depending on the instrument used) and needs to be run for 24 hours, to ensure the material is completely frozen, before the vacuum is switched on. Oil changes have to be frequent, especially when organics are likely to be drawn out of the sample and this slows down the freeze-drying process. The presence of other materials within the freeze-dryer can also affect the behaviour of the process and can again slow down drying. Hence, freeze-drying is an expensive and time-costly technique.

During oven-drying, temperatures were kept deliberately low, at 40^{o}C, so that genetic information and seed viability would not be compromised. A higher temperature would possibly have been more effective at removing the naphthalene, but then more damaging to the specimen. Both the specimen and the herbarium sheet are comprised of cellulose, which is susceptible to thermal degradation. Heating the specimens can also damage the mounting materials used (i.e. glue or tape) and the attachment of the label. Heating also requires energy and depending on the size of the oven, could be very timely and therefore not cost-effective.

Herbaria are now known to be hazardous areas in which to work and hence fume cabinets are often readily available and could be utilised for air-drying without additional expense. The use of fume cabinets in air-drying is advisable as the hazardous pesticides, including mercuric chloride, will be drawn off safely and removed from the working environment. Although, in principle, air-drying poses no threat to the specimens, the airflow needs to be carefully controlled to avoid damaging fragile specimen parts.

12 Conclusions

A number of key conclusions can be drawn from the research:

- The decontamination tests have shown that air-drying is more efficient at removing naphthalene than either freeze-drying or oven-drying. It is also the most cost-effective, and the least damaging to the specimen.

- It is necessary to maintain a continuous air flow over the sample to remove the desorbed naphthalene. The freeze-drying and oven-drying tests both demonstrated how some paper types readily absorb naphthalene during the drying process, if the naphthalene is allowed to build up around the sample.

- There is some evidence to suggest that the naphthalene is not only absorbed at the surface of the specimen sheets, but also held within the body of the paper. Although the naphthalene moves to the surface during the drying process, this has implications for the optimum duration of the drying process – longer periods of time may be required to reduce the overall naphthalene concentration in the specimen sheets.

- The paper type clearly influences the efficiency of naphthalene removal. The thin, unfinished and hence porous, papers are better disposed to losing naphthalene than the heavier, finished and coated papers. Their porous structure allows the naphthalene to remain more mobile. This gives important information about which specimen sheets are more likely to be successfully decontaminated by the air-drying procedure and can inform the selection and prioritisation process.

PART IV: FINAL CONCLUSIONS AND RECOMMENDATIONS

The presence of large amounts of hazardous biocide residues within herbaria is a legacy from past conservation treatments affecting many museums and institutions across the world. The problems that this can cause, from a health and safety perspective, is exacerbated by the paucity of historic information regarding the treatments applied to the specimens in the past.

For an institution legally required to protect its staff, visitors, volunteers and researchers, the detection and removal of hazardous material from the herbarium environment is a top priority. Unfortunately, many herbaria are vast, often containing hundreds of thousands of specimens (the AC-NMW herbarium contains over 250,000 higher plant specimens), making such a task difficult – both costly and time-consuming – and impossible to achieve in many herbaria.

Research has demonstrated that the AC-NMW collection is heavily contaminated with mercuric chloride and naphthalene residues, two of the most common biocides used historically and ubiquitous in many collections across the world.

A rapid and cost-effective screening method for identifying which herbarium sheets within a collection have been treated historically with mercuric chloride has been developed. The use of a hand-held UV-A lamp to identify the presence of fluorescence, as chemical markers of contamination, will be an accessible and affordable methodology for the majority of museums across the world. It will provide a simple and economical means to prioritise which collections require immediate re-mounting, and will inform the implementation of safe, standard procedures to protect personnel and visitors. It will also enable the removal of a large amount of hazardous chemical from the herbarium environment.

The presence of several emission wavelengths in the fluorescence observed, giving rise to the different coloured areas on the sheet, may offer the opportunity to further develop this screening method to allow the estimation of contaminant level. Further research is needed to establish if there is a direct correlation between the colour of fluorescence observed and the Hg(I) concentration within.

The removal of hazardous biocides through decontamination, whilst maintaining the integrity of the specimen, is particularly challenging. Laser ablation, although showing some promise in removing inorganic residues from the surface (such as lead, sulphur and chlorine), the technique is both expensive and time-consuming, and may not remove contaminants absorbed beneath the surface layers.

The most efficient and cost-effective method for the safe removal of naphthalene from the collection has been demonstrated to be air-drying, with a low, continual air flow being maintained over the sample. A maximum loss of 79% naphthalene (by weight) was observed using this method over a 48 hour period. This is a simple and realistic method, considering the size of the collections involved, and the extent to which naphthalene has been incorporated into herbaria. The desorption

of naphthalene, however, is not straightforward. Even with a continual air flow over the samples, the surface concentration of naphthalene was observed to increase after the initial 48 hours of treatment. It is believed that this is due to the naphthalene held within the body of the paper being mobilised to the surface, rather than from reabsorption. Further research, however, is needed to understand the kinetics of the process and to determine an effective protocol for optimum success.

The presence of hazardous biocide residues within herbaria is also a concern for the integrity of the collection as an important research resource. The presence of certain biocide residues on zoological material has dramatically reduced, or completely hindered, the extraction of genetic information. Although the biocide residues within herbaria are mainly associated with the mount sheets, a quantity of biocide remains on the specimen. Further research is needed to determine whether the biocide residues will affect the extraction of genetic information in a similar way to that observed in animals.

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APPENDIX 1

1 Legislation[1]

The work place can be a source of a variety of different substances (e.g. chemicals, fumes, dusts, fibres) which may have a harmful effect on the workers' health. These are called hazardous substances (HSE 2005). Work place exposure to hazardous substances, including historic pesticide residues, must be monitored and controlled, and is the responsibility of the employer. The primary legislation concerned with health and safety in the work place in the UK is the Health and Safety at Work Act (1974). The Act states that the employer has a responsibility towards the health and safety of their employees and of the general public who may be affected by the employer's activities. The Health and Safety at Work Act (1974) is in addition to existing acts, including the Factory Acts of 1833.

1.1 Restrictions on Use of Biocides

Prior to 1986, there were no statutory schemes for the control of pesticides in the UK. A voluntary scheme, the Pesticides Safety Precautions Scheme (PSPS) existed from c.1975 to 1986 for non-agricultural pesticides. No records were found giving clearance for the use of mercuric chloride, barium fluorosilicate or lead arsenate as non-agricultural pesticides, during this period. Arsenic trioxide was recorded in a small number of anti-fouling paints, brought under the regulations in 1987 (HSE, 2003). The statutory Control of Pesticide Regulations (COPR) replaced the PSPS in 1986. The continued use of mercuric chloride, barium fluorosilicate and lead arsenate as non-agricultural pesticides after 1986 is illegal[2].

The Marketing and Use Directive banned anti-fouling paints containing arsenic trioxide in 1989 under Directive 89/677/EEC[3]. This was enacted in Great Britain by revoking the COPR approvals for the arsenic trioxide products, with approvals for advertisement and sale being withdrawn in 1989, and approvals for supply, storage and use being revoked in 1990. This active ingredient is not used in any other non-agricultural pesticide approved under the regulations, and therefore its use as a non-agricultural pesticide has been illegal in the UK since 1990. There are records of mercuric chloride having been approved by the Department for Environment, Food, and Rural Affairs (DEFRA) under COPR, as an agricultural fungicide, but its use was banned in 1992 under Directive 79/117/EEC3.

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1 The majority of the data presented has been accumulated courtesy of the Welsh National Poisons Unit, Llandough Hospital, Cardiff, Wales. The National Centre for Environmental Health. Medline Plus 8/9/2005 and the Centres for Disease Control and Prevention. Ellenhorn, M. J., Schonwald, S., Ordog, G.& Wasserberger (1997). Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning. Baltimore, Williams & Wilkins. IPCS Inchem http://www.inchem.org/pages/search.html

2 Council Directive 79/117/EEC dated 21 December 1978 prohibits the placing on the market and the use of plant protection products containing certain active substances which, even if applied in an approved manner, could give rise to harmful effects on human health or the environment. Pesticide Safety Directorate Web site http://www.pesticides.gov.uk/blue%5Fbook/annex%5Fb/banned%5Fand%5F%20non%5Fauthorised.html

3 31989L0677R(02) corrigendum to: Council Directive 89/677/EEC of 21 December 1989 amending for the eighth time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the member states relating to restrictions on the marketing and use of certain dangerous substances and preparations Official Journal L 250 , 23/09/1999 P. 0015 http://europa.eu.int/eur-lex/en/search/search_lif.html

1.2 COSHH

The Health and Safety Executive/Commission act as the regulatory authority in the UK for biocides and non-agricultural pesticides by administering regulatory schemes. In 1988 the HSC brought before Parliament a document stating the regulations associated with The Control of Substances Hazardous to Health (COSHH). COSHH regulations came into force on 1st October 1989 and are an EEC-driven initiative. The regulations were aimed at imposing an influence over almost every organization in the UK, raising awareness of working with hazardous chemicals and ensuring that both employers and employees were fully aware of their responsibilities prior to using any chemical that could impact upon the health of the user or of an individual exposed through sharing work areas.

COSHH has provided the exposure limits of all registered chemicals that are classed as hazardous substances. These are listed in EH/40 a publication updated regularly by the HSE. COSHH also provides information on the monitoring of air quality of historic substances and provides guidelines on biological monitoring of staff. COSHH stresses that the employer is fully responsible for providing protection, information and training to any personnel working in connection with the employer's duties that may involve exposure to classified hazardous substances.

Control of Lead in the Workplace

Lead has been assigned a law, separate to the COSHH regulations. These regulations were put in place to control lead in the working environment and were called the Control of Lead at Work Regulations 2002. These guidelines were made on the 24th October 2002 and came into force on the 21st November 2002. This was deemed essential, as lead is an extremely toxic element that accumulates in the body and can reach damaging levels in the blood.

1.3 Occupational health exposure

Table A1 summarises the numerous and varied acronyms associated with occupational health exposure. As there are so many variations on what is often the same exposure reference, it has become increasingly difficult for institutions to understand how to apply each limit, and therefore comply with the different guidelines for each individual situation.

Workplace Exposure Limit (WEL)

The occupational exposure limits set out previously in EH40/2002 are for use in the amended COSHH regulations 2002. However, the OEL, MEL and former occupational exposure standards have since been withdrawn and replaced with one single occupational limit, a workplace exposure limit (WEL). A WEL will provide a value relating to the maximum concentration of an airborne substance, averaged over a reference period, to which employees may be exposed by inhalation (HSE 2005).

Table A1 Acronyms and definitions associated with toxicology and exposure values

Acronym	Reference Limit	Region
BOELV	Binding Occupational Exposure Limit Values	EC
BLV	Biological Limit Value	EC
BMGV	Biological Monitoring Guidance Values	UK
IDLH	Immediately Dangerous to Life or Health	US
IOELV	Indicative Occupational Exposure Limit Values	EC
OEL	Occupational Exposure Limit	US, EC
OES	Occupational Exposure Standard	US
MEL	Maximum Exposure Limit	UK
PEL	Permissible Exposure Limit	US
REL	Recommended Exposure Limit	US
STEL	Short Term Exposure Limit	EC, UK, US
TWA	Time Weighted Average	EC, UK, US
TLV	Threshold Limit Value	US
WEL	Workplace Exposure Limit	UK
Ceiling	Ceiling exposure limit…should not be exceeded, unlike previous OES	UK, US, EC

2 Toxic effects of NMW pesticides

The toxic effects of the NMW herbarium pesticides are summarised in Table A2.

Table A2 Summary of toxic effects of NMW pesticides

	Mercuric chloride	Arsenic trioxide	Lead arsenate	Barium fluoro-silicate	Naphthalene
Reproductive problems	Foetal damage and genetic mutations, Kidney damage	Malformations of mice/rat off-spring	Reproduction problems in men and women. Prenatal expo-sure can cause premature birth, low birth weight, decreased mental ability, learning difficulties, and slow growth.	No evidence at this point	
STEL (mg/m³)	0.015	0.002	Presently unavail-able		80 (15ppm)
LTEL (mg/m³)	0.025	0.01	0.15	0.5	53 (10ppm)
New values (mg/m³)	0.02 WEL	0.1 MEL	0.1 Ceiling		
Routes of entry into system	Absorption through skin, inhalation, ingestion	Absorption through skin, inhalation, inges-tion	Ingestion, inhala-tion	Inhalation and ingestion	Absorption through skin, inha-lation, ingestion.
Short term effects	Eye irritation, burns, damage, CNS dam-age, lung irritation, coughing, possible pulmonary oedema.	Hoarse voice, irritation to nose, eyes, skin and mucous mem-branes, nausea, vomiting, diar-rhoea, weakness, loss of appetite, coughing, chest pain, giddiness, headache, breath-ing difficulty.	Cough sore throat, sore eyes and red skin, vomit-ing, diarrhoea and abdominal pain, oedema, conjunctivitis and liver enlarge-ment.	Gastroenteritis, loss of deep reflexes, mus-cular paralysis, recovery often rapid.	Stomach prob-lems, nausea, vomiting, diar-rhoea abdominal pain, Irritation of eyes, nose and throat.
Long term effects	Sore gums, shakes, memory loss, weak-ness, loss of teeth, poor appetite.	Heart, kidney brain, lung and gastrointestinal tract damage. Eventual skin, bone marrow and peripheral nervous system damage.	CNS damage and death	Repeated or chronic exposures have been reported to cause osteoscle-rosis, as with fluoride.	Fever, pain when urinating, low urine output Convulsions Short-ness of breath increased heart rate low blood pressure jaundice, headache drowsi-ness confusion coma.
Carcinogen	Possible human carcinogen	Class A Oncogen	Class A Oncogen	No evidence	In animal studies it causes cancer not yet verified in humans, although suspected.

Summary of NMW pesticides and their current status

Arsenic trioxide (As_2O_3) (CAS No: 1327-53-3. Mwt.197.8)

Alternative names: *arsenous oxide, arsenic oxide, arsenic (III) oxide, arsenous oxide anhydride, white arsenic, arsenous acid anhydride*.

All arsenic compounds are considered to be extremely poisonous and carcinogenic.

*HSC/E plans to review the limit for this substance

Lead arsenate ($PbHAsO_4$) (CAS No: 7784-40-9. Mwt. 347.1)

Alternative names: *Arsenic acid, lead salt, acid lead arsenate, dibasic lead arsenate*.

Lead arsenate is harmful by inhalation and if swallowed, also danger of cumulative effects.

Mercuric chloride ($HgCl_2$) (CAS No: 7487-94-7 Mwt. 271.5)

Alternative names: *Corrosive sublimate, mercury dichloride, mercury (II) chloride*

Mercury salts are very toxic by inhalation, in contact with skin, and if swallowed. There is also danger of cumulative effects.

Barium fluorosilicate ($BaSiF_6$) (Cas No. 17125-80-3. Mwt.279.42)

Alternative names: *Barium fishmoth poison, fishmoth bait, silverfish bait, barium silicofluoride, fluorosilicic acid salts, barium fluorosilicate, barium hexafluorosilicate(2-) hexafluorosilicate(2-) barium (1:1), silicate(2-), hexafluoro-, barium.*

Barium compounds are harmful if inhaled or swallowed. There is no specific information relating to the insecticide barium fluorosilicate.

Addendum to EH40 2005: This was produced in July 2006 as the Health and Safety Commission agreed to revise the Workplace Exposure Limit (WEL) for respirable crystalline silica from 0.3 to 0.1. HSC/E plans to keep this limit under review. This will be in place by 01/10/2006.

Naphthalene ($C_{10}H_8$) (CAS No 91-20-3. Mwt.128, Mpt. 80.3°C)

Alternative names: *Camphor tar, white tar, tar camphor, moth balls*.

Naphthalene is not supported as a pesticide by Commission Regulation (EC) No 1112/2002, as no safe level has been set, and the occupational exposure standard has been withdrawn. It is still classified as a suspected carcinogen.

The UK Advisory Committee on Toxic Substances has expressed concern that OELs may not be adequately protective because of doubts that the limit was not soundly-based. These OELs were included in the published EH/40 2002 list and its 2003 supplement, but were omitted from the published 2005 list.

APPENDIX 2

Material Safety Data Sheets

All MSDS taken from The Physical and Theoretical Chemistry Laboratory , Oxford University, Chemical and Other Safety Information, http://ptcl.chem.ox.ac.uk/MSDS/

For risk and safety phrases mentioned in this document see p. 189

Safety data for ARSENIC (III) OXIDE

General

TOXIC

Synonyms: arsenic oxide, arsenous acid, arsenous oxide, arsenic trioxide, white arsenic

Molecular formula: As_2O_3 CAS No: 1327-53-3 EINECS No: 215-481-4

Physical data

Appearance: white powder

Melting point: 315°C

Boiling point: 457°C

Vapour density:

Vapour pressure:

Density (g cm^{-3}): 3.738

Flash point:

Explosion limits:

Autoignition temperature:

Water solubility:

Stability

May decompose on exposure to moist air or water. Incompatible with oxidizing agents, heat, metals. Reaction with acids generates arsine, which is highly toxic.

Toxicology

Highly toxic. May be fatal if swallowed. May cause allergic respiratory reaction. May act as a carcinogen - inorganic arsenic is a known cancer hazard. May cause skin or eye burns. Typical TLV/TWA 0.2 mg/m^3.

Toxicity data

ORL-MAN LDLO 29 $mg\ kg^{-1}$

ORL-RAT LD50 14 $mg\ kg^{-1}$

SCU-MUS LD50 10 $mg\ kg^{-1}$

ORL-RBT LD50 20 $mg\ kg^{-1}$

Risk phrases

R28 R34 R45.

Transport information

Hazard class: 6.1. Packing group: II

Personal protection

Gloves, safety glasses, good ventilation. Handle as a carcinogen.

Safety phrases

S45 S53.

Safety (MSDS) Data for MERCURY (II) CHLORIDE

TOXIC, CORROSIVE, ENVIRONMENTAL HAZARD

General

Synonyms: mercury (II) chloride anhydrous, mercuric chloride, mercury dichloride, mercury bichloride, mercuric bichloride, dichloromercury, mercury perchloride, abivit B, fungchex, corrosive sublimate, sulem

Use: embalming chemical, disinfectant, photographic intensifier, fungicide, insecticide, steel and iron etchant

Molecular formula: $HgCl_2$

Mwt 271.52

CAS No: 7487-94-7

EC No: 231-299-8

Annex I Index No: 080-010-00-X

Physical data

Appearance: white crystalline powder

Melting point: 277°C

Boiling point: 302°C

Vapour density: 8.7 (air = 1)

Vapour pressure:

Density (g cm-3): 5.44

Flash point:

Explosion limits:

Autoignition temperature:

Water solubility: moderate

Stability

Stable, but moisture sensitive and light sensitive - decomposes in sunlight. Incompatible with strong acids, ammonia, carbonates, metallic salts, alkalies, phosphites, phosphates, sulfites, sulfates, arsenic, antimony, bromides.

Toxicology

Poison. May be fatal if inhaled, swallowed or absorbed through the skin. Long-term exposure, even at low levels, may lead to impaired memory, build-up of mercury in body organs, loss of appetite, loose teeth and other effects. Note low LD50 values below. Severe irritant. Corrosive. Typical TLV/TWA 0.05 mg m^{-3}

Toxicity data

ORL-RAT LD50 1 mg kg^{-1}

IPR-MUS LD50 5 mg kg^{-1}

SCU-RAT LD50 14 mg kg^{-1}

ORL-WMN TDLO 50 mg kg^{-1}

ORL-HMN LDLO 29 mg kg^{-1}

ORL-RBT LD50 40 mg kg^{-1}

Risk phrases

R24 R25 R28 R34 R48 R50 R53.

Environmental information

Very toxic to aquatic organisms - may cause long-term damage in the environment.

Transport information

UN No 1624. Hazard class 6.1. Packing group II.

Personal protection

Safety glasses, gloves, good ventilation.

Safety phrases

S36 S37 S39 S45 S60 S61

Safety (MSDS) Data for *O*-XYLENE

HARMFUL

General

Synonyms: *Ortho*-xylene,1,2-dimethylbenzene

Molecular formula: C_8H_{10}

CAS No: 95-47-6

EC No: 202-422-2

Annex I Index no: 601-022-00-9

Physical data

Appearance: colourless liquid

Melting point: -24°C

Boiling point: 144°C

Vapour density: 3.7

Vapour pressure: 7 mm Hg at 20°C

Specific gravity: 0.87

Flash point: 32°C (closed cup)

Explosion limits: 1.1 % - 7 %

Autoignition temperature: 463°C

Stability

Stable. Incompatible with oxidizing agents. Flammable. Hygroscopic.

Toxicology

Harmful if inhaled or absorbed through the skin. Narcotic. May cause lung irritation, chest pain or fatal oedema. May impair fertility. Skin irritant. Typical STEL 150 ppm.

Toxicity data

IPR-MUS LD50 1.5 ml kg^{-1}

Risk phrases

R10 R20 R21 R38.

Personal protection

Safety glasses, adequate ventilation.

Safety phrases

S25.

Safety (MSDS) data for CARBON DISULPHIDE

TOXIC, HIGHLY FLAMMABLE

General

Synonyms: carbon bisulphide, carbon bisulfide, carbon disulfide, carbon sulfide, dithiocarbonic anhydride, NCI-C04591, weeviltox, sulphocarbonic anhydride

Use: Disinfectant, insecticide, bactericide, preservative, solvent, chemical reagent

Molecular formula: CS_2

CAS No: 75-15-0

EC No:

Physical data

Appearance: colourless to light yellow liquid with an unpleasant odour

Melting point: -112°C

Boiling point: 46°C

Vapour density: 2.67 (air = 1)

Vapour pressure: 300 mm Hg at 20°C

Density (g cm^{-3}): 1.26

Flash point: -30°C

Explosion limits: 1 - 50%

Auto ignition temperature: 90°C

Water solubility: slight

Stability

Stable. Extremely flammable. Highly volatile. Note low flash point and very wide explosion limits. Protect from heat, friction, shock, sunlight. Reacts violently with fluorine, azide solutions, zinc dust, liquid chlorine in the presence of iron. Incompatible with strong oxidizing agents, azides, aluminium, zinc, most common metals, nitrogen oxides, chlorine, fluorine, hypochlorites.

Toxicology

Poison - may be fatal if swallowed or inhaled. Serious health hazard, affecting the CNS. Readily absorbed through the skin. Sufficient material may be absorbed through the skin to be fatal. May cause reproductive damage. Chronic exposure may cause liver, kidney and CNS damage, or impaired vision. Causes burns. Severe eye and respiratory irritant. Skin irritant. Typical PEL 7 ppm.

Toxicity data

(The meaning of any abbreviations which appear in this section is given here.)

IHL-HMN	LCLO	400ppm/30m
IPR-GPG	LDLO	400mgkg-[1]
IHL-MAM	LCLO	2000ppm/5m
ORL-RAT	LD50	3188mgkg:-[1]
ORL-MUS	LD50	2780mgkg-[1]

ORL-RBT LD50 2550mgkg-[1]

IHL-MUS LC50 10000 mg/m^3/2h

Risk phrases

R12 R23 R24 R25.

Transport information

Major hazard class 3. Subsidiary risk 6.1. UN No 1131. Not permitted on passenger or commercial cargo planes.

Personal protection

Because of its low ignition temperature vapours of this material may ignite if reaching hot objects such as hot water pipes, light bulbs, hot plates, heating mantles etc. Vapour may flow a considerable distance to a source of ignition. Wear safety glasses and Viton or PVA gloves, and use good ventilation.

Safety (MSDS) data for NAPHTHALENE

TOXIC

General

Synonyms: albocarbon, mighty 150, mighty rd1, moth flakes, NCI-C52904, white tar, naphthalin, naphthene, camphor tar, tar camphor, moth balls

Molecular formula: $C_{10}H_8$

CAS No: 91-20-3

EC No: 202-049-5

Physical data

Appearance: white crystals

Melting point: 77°C

Boiling point: 218°C

Specific gravity: 1.14

Vapour pressure: 1 mm Hg at 20°C

Vapour density: 4.4 g/l

Flash point: 88°C

Explosion limits: 0.9 - 5.9%

Auto ignition temperature:

Stability

Stable. Flammable - avoid sources of ignition. Incompatible with oxidising agents. Heat-sensitive. Sublimes slowly at room temperature.

Toxicology

May cause irritation. Toxic by inhalation or ingestion. TLV 10 ppm. Sensitiser. Possible carcinogen.

Toxicity data

ORL-CHD LDLO 100 mg kg-1

UNR-HMN LDLO 29 mg kg-1

ORL-MUS LD50 533 mg kg-1

IVN-MUS LD50 100 mg kg-1

ORL-RBT LD50 3000 mg kg-1

Irritation data

EYE-RBT 100 mg/mld

SKN-RBT 495 mg open mld

Risk phrases

R20 R21 R22 R36 R37 R38 R43 R45.

Personal protection

Safety glasses. Use efficient ventilation.

Safety phrases

S16 S26 S36 S37 S39 S45.

Safety (MSDS) data for SODIUM SILICATE

HARMFUL

General

Synonyms: silicic acid sodium salt, water glass, sodium water glass, soluble glass, silicate of soda, silicon sodium oxide, sodium orthosilicate, sodium sesquisilicate, sodium silicate glass, agrosil S, barasil S, britesil, carsil 2000, chemfin 60, chemsilicate, crystal 79, crystal 96, ineos 140, inosil Na 4237, portil A, pyramid 8, vitrosol N40, ZhS 3, very large number of further trade names

Molecular formula: Na_4O_4Si

CAS No: 1344-09-8

EC No: 239-981-7

Physical data

Appearance: colourless liquid as usually supplied (solution)

Melting point:

Boiling point: ca. 102°C for a 40% aqueous solution

Vapour density:

Vapour pressure:

Specific gravity: approximately 1.3 for a ca. 40% solution

Flash point:

Explosion limits:

Auto ignition temperature:

Stability

Stable. Incompatible with acids, most metals, many organic materials.

Toxicology

Harmful by ingestion. Corrosive - may cause burns through skin or eye contact. Very destructive of mucous membranes.

Risk phrases

R22 R34.

Transport information

Non-hazardous for air, sea and road freight.

Personal protection

Safety glasses, gloves.

Safety phrases

S23 S24 S25 S26 S27 S36 S37 S39.

Safety (MSDS) data for NITRIC ACID

TOXIC, CORROSIVE

General

Synonyms: azotic acid, aqua fortis

Molecular formula: HNO_3

CAS No: 7697-37-2

EC No: 231-714-2

Physical data

Appearance: colourless liquid with a choking odour

Melting point: -42°C

Boiling point: 121°C (69% boils at ca. 86°C)

Specific gravity: 1.41

Vapour pressure: 62 mm Hg at 20°C (68%)

Stability

Stable. Strong oxidiser. Substances to be avoided include strong bases, strong reducing agents, alkalis, most common metals, organic materials, alcohols, carbides. Corrodes steel. Light-sensitive.

Toxicology

May be fatal if swallowed or inhaled. Extremely corrosive. Contact with skin or eyes may cause severe burns and permanent damage. TLV 2 ppm. OES long-term 5 mg/m^3

Toxicity data

IHL-RAT LC50 244 ppm (NO_2)/30m

ORL-HMN LDLO 430 $mg\ kg^{-1}$

Risk phrases

R8 R23 R24 R25 R34 R41.

Transport information

UN No 2031. Packing group II. Hazard class 8.0. Transport category 2.

Personal protection

Safety glasses or face mask, gloves. Fume cupboard.

Safety phrases

S23 S26 S36 S37 S39 S45.

Risk Phrases

R8 Contact with combustible material may cause fire

R10 Flammable

R12 Extremely flammable

R20 Harmful by inhalation

R21 Harmful in contact with skin

R22 Harmful if swallowed

R23 Toxic by inhalation

R24 Toxic in contact with skin

R25 Toxic if swallowed

R26 Very toxic by inhalation

R28 Very toxic if swallowed

R34 Causes burns

R36 Irritating to eyes

R37 Irritating to respiratory system

R38 Irritating to skin

R41 Risk of serious damage to eyes

R43 May cause sensitisation by skin contact

R45 May cause cancer

R48 Danger of serious damage to health by prolonged exposure

R50 Very toxic to aquatic organisms

R53 May cause long-term adverse effects in the aquatic environment

Safety Phrases

S16 Keep away from sources of ignition - No smoking

S23 Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer)

S24 Avoid contact with skin

S25 Avoid contact with eyes

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S27 Take off immediately all contaminated clothing

S36 Wear suitable protective clothing

S37 Wear suitable gloves

S39 Wear eye/face protection

S45 In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)

S53 Avoid exposure - obtain special instructions before use

S60 This material and its container must be disposed of as hazardous waste

S61 Avoid release to the environment. Refer to special instructions/safety data sheet

Suppliers

Blue Nitrile disposable gloves	Fisher Scientific UK, Bishop Meadow Road,Loughborough, Leicester. Aldrich Chemical Company, The old Brickyard, New Road, Gillingham, Dorset, SP8 4XT.
Laboratory Coat	Fisher Scientific UK, Bishop Meadow Road,Loughborough, Leicester. Aldrich Chemical Company, The old Brickyard, New Road, Gillingham, Dorset, SP8 4XT.
Mercury Vapour Respirator	Fisher Scientific UK, Bishop Meadow Road,Loughborough, Leicester. Aldrich Chemical Company, The old Brickyard, New Road, Gillingham, Dorset, SP8 4XT.
Eye protection	Fisher Scientific UK, Bishop Meadow Road,Loughborough, Leicester. Aldrich Chemical Company, The old Brickyard, New Road, Gillingham, Dorset, SP8 4XT.
Air One Fume Cabinet *C100 Size B charcoal Filter*	SafeLab Systems Unit 29, Linx Crescent Weston Super-mare BS24 9BZ
Naphthalene *Nitric Acid* *Hydrochloric Acid* *Phluroglucinol*	Sigma-Aldrich Company Ltd Fancy Road, Poole Dorset, BH12 4QH.

o-Xylene	Prolabo12, rue Pelee F75011, Paris
Sodium silicate Carbon disulfide *Activated Carbon* *Whatman No 1 filter paper*	Fisher Scientific UK Ltd Bishop Meadow Road Loughborough, Leicestershire LE11 5RG
Archipress® Transparent Polyester Vacuum Pouches *Oxygen Free Packaging*	Conservation by Design Timecare Works, 5 Singer Way, Woburn Road Industrial Estate, Kempston, Bedfordshire, MK42 7AW.
Copper wire	Fisher Scientific UK Ltd Bishop Meadow Road Loughborough, Leicestershire LE11 5RG
Parafilm	Sigma-Aldrich Company Ltd Fancy Road, Poole Dorset, BH12 4QH.
Tenax TA®	Sigma-Aldrich Company Ltd Fancy Road, Poole Dorset, BH12 4QH

APPENDIX 3

Publications and Presentations of this Research (2002 - 2012)

Publications

PUREWAL, V., COLSTON, B. AND RÖHRS, S. (2007) In *The XI International Conference on Particle Induced X-Ray Emission and its Analytical Applications.*, Vol. 1 (Eds, Miranda, J., Ruvalcaba-Sil, J. L. and de Lucio, O. G.) Universidad Nacional Autónoma de México, 2007, Puebla, Mexico, pp. D3 1-4.

PUREWAL, V., COLSTON, B. AND RÖHRS, S. (2008). Developing a simple screening method for the identification of historic biocide residues on herbarium material in museum collections. *X-Ray Spectrometry* **37** (2), 137–141.

Conference Presentations

PUREWAL, V., COLSTON, B. AND MORGAN, D. (2009). Recognition of the relationship between a cellulose substrate and historic biocides applied to herbaria over time. *Bridging Continents – New initiatives and perspectives in natural history collections*. The Society for the Preservation of Natural History Collections, *SPNHC 2009*.

COLSTON, B. AND PUREWAL, V. (2008). Development of a novel approach to the identification of historic herbarium biocides. *Conservation Science Annual* at the *2008 Eastern Analytical Symposium*, November 2008, New Jersey (INVITED).

PUREWAL, V. AND COLSTON, B. (2008). New approaches to managing contaminants in herbaria.. *Society for the Preservation of Natural History Collections Annual Conference*, Berlin.

PUREWAL, V., COLSTON, B. AND RÖHRS, S. (2007). The identification of historic pesticide and fungicide residues present on herbarium material housed within the National Museum Wales. *Proceedings of the 11th International Conference on Particle-induced X-Ray Emission and its Analytical Applications,* PIXE 2007.

PUREWAL, V. AND COLSTON, B. (2006). OLD POISONS – New Approaches (2006). *Society for the Preservation of Natural History Collections Annual Conference* Albuquerque, New Mexico, May 2006.

PUREWAL, V. AND COLSTON, B. (2005).The Identification of Hazardous Pesticide and Fungicide Residues Present on Herbarium Mount Paper. *Metals in Paper*, Rome, February 2005.